Neural representations of sensory discrimination

Neuronale representatie van zintuigelijke waarnemingen

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Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
Op gezag van de rector magnificus

Prof.dr. H.G. Schmidt en
volgens besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden op
woensdag 9 oktober 2013 om 9:30 uur

door

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Illustration on cover: *Blind men and the elephant*

*The blind men and the elephant* is an old Indian parable that illustrates the inaccessibility of the nature of Truth. It has been adopted across many religions and cultures and interpreted in various ways. The story goes that a group of blind men attempt to touch the elephant in order to find out exactly what it is. As the different men touch different parts of the elephant’s body, they come to different conclusions as to the true nature of the object of inquiry. For example, one man touches the leg and describes the object as a pillar, while another touches the tail and says it is a rope. Again another feels the trunk and deduces it is a tree branch, the ear feels like a hand fan, the belly a wall, and the tusk a solid pipe. Conflict between the men and their interpretations ensue.

This illustration seems fitting to the present thesis as a way to not only exemplify the power of neuroscience, but also to admit its limitations. Whereas we know that our subjective experience is true, it may not be the totality of truth. In science we attempt to discover how something works, but our limitations are elucidated through only what is possible to perceive.

Although different versions of the parable resolve the men’s conflict in different ways, I choose to see a scientist’s ending to the story, where they stop arguing, begin listening, and collaborate their experiences to come to know the “whole” elephant.

*voor mijn ouders*

*for my parents*
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CHAPTER I

General Introduction
1.1 Learning and memory

1.1.1: Science as objective inquiry

Although mankind’s presence on this planet has been comparatively short relative to that of many species sharing it with us, we have built a society that has and is flourishing. Over time our grasp on our surroundings has increasingly become more complex and through science and technology we have shaped our environment as much as it has shaped us. The discovery of fire, the agricultural revolution, the advance of medicine, and the harnessing of energy are some examples of a species aiming to take control of its environment for meeting its goal of survival. These advances are built upon a foundation of knowledge that has helped facilitate population growth. The theory of natural selection and how environments interact with populations seems intuitive now, but was not made explicit with empirical observational evidence until ideas of Charles Darwin and Alfred Wallace arrived in 1859 and Gregor Mendel’s experiments in plant hybridization in 1866. Darwin described how wet and dry seasons in the Galapagos islands favored finch populations with different beaks, an example of how the environment can shape a species’ population and survival. Mendel laid the groundwork for modern genetics through experimentation with peas. These figures brought knowledge to society about how our environment and genetics are interrelated.

Learning and memory is a major catalyst for allowing a species to take control of its environment. It is the foundation from which conscious living arises and is therefore pivotal for our species interpretation for quality of life.

Broadly, cognitive neuroscientists might define learning as the feature by which we alter our skills, change our dispositions, add to our knowledge and generally benefit or suffer from an experience. Memory is the expression of that learning, as exemplified in alterations of our performance across a broad range of daily activities [1].

Through human history, examples of learning are ever-present in everything from speaking to riding a bike. Therefore, much of learning takes place when an event or experience from the outside world is processed through our senses first, and later perceived and interpreted by our minds. A neuroscientist’s basis for understanding the mind is then through understanding the structure and function of the brain.

Understanding the mind requires the ability of learning and memory and all the facets of this process can be empirically investigated through the guise of neuroscience. However, a prominent existentialist, Karl Jaspers, reminds us: “If we make ourselves into the object of our thinking, we ourselves become as it were the Other, and yet at the same time we remain a thinking I, which thinks about itself but cannot aptly be thought as an object because it determines the objectness of all objects” [2]. This statement renders true in reference to philosophical inquiry, but Ramon y Cajal, a pre- eminent neuroscientist, explains that science
cannot hope to solve ultimate causes, that is, the foundation hidden below the appearance of phenomenon in the universe [3]. By using the scientific method and attempting to measure precisely, we can demonstrate the “how” of the brain, rather than the “why”. This endeavor has value, and just like learning to ride a bike, each experiment can build upon the previous. Just as an individual builds upon each experience to learn new things, a society can build upon discoveries that cause a new way of thinking for a people.

1.1.2: Classical conditioning and the cognitive map theory

The Russian physiologist named Ivan Pavlov is an example of someone who built upon his experiences in the lab to cause a paradigmatic shift in behavioral, psychological, and neuroscientific research in 1927. With the initial aim of studying the digestive system, he found that dogs would naturally salivate if presented with food. If he delivered a stimulus such as the ringing of a bell, electric shock, or visual stimuli paired with the presentation of food, the dog would salivate to the food only but associate the food with the stimulus. Eventually the dog would reflexively make the association between the stimulus and food and salivate at the presentation of the stimulus alone [4]. This process is termed a conditioned reflex where the dog’s initial salivation to food is the unconditioned response (UR) and the food is the unconditioned stimulus (US) prior to the association being made. Once the association is made with the bell, shock, or other stimuli it is called the conditioned stimulus (CS) and the response to the CS is called the conditioned response (CR) (see figure 1.1A).

This conditioning, also known as classical conditioning, was followed by the discovery and description of operant conditioning by Burrhus Skinner, where associations are used for the modification of voluntary behavior; for example a rat could press on a lever, receive food, and make the association that by pressing this lever it can receive food through a dispenser (Skinner, B.F. 1938). Operant conditioning is distinguished from classical conditioning in that the conditioned response is voluntary rather than reflexive. These works catapulted the scientific community into a new “behaviorist” way of thinking; in which all behaviors were interpreted as stimulus-response associations.

However, it wasn’t until Edward Tolman challenged the notion that all learning could be reduced to stimulus-response mechanisms in his paper “Cognitive maps in rats and men” in 1948, [5]. He used rats and a maze with three possible routes, each of varying distances to the reward. The rats first freely explored all of the possible routes of the maze without any contingency. After this learning period, when the rat was free to choose any path, it always took path one, the most direct path to the box. When path one was blocked the rat could adjust its strategy without prior response-reward training and would, again, take the most direct route available, path two. Finally when route one and two were blocked, he would then take a third untrained approach, the most circuitous and only available route, route three.
Classical conditioning, discovered by Ivan Pavlov, requires an unconditioned stimulus paired with a neutral stimulus that, upon achieving a conditioned response is termed the conditioned stimulus. In classical conditioning the response associated with the stimulus is reflexive, such as blinking your eye. In operant or associative conditioning the response is voluntary, such as licking for a reward.

Tolman hypothesized that the rat had general knowledge about the overall maze from its previously unrewarded exploration, and that therefore such novel responses could not have been acquired through previously rewarded behaviors [1]. He further provided evidence that a rat can learn where a reward is located, regardless of the animal’s egocentric positioning. This place learning is in contrast to learned behavioral responses from repeated experience to get a reward, known as response learning. Place learning as opposed to response learning asserted two important contrasting aspects to behaviorist theory; (1) it involves knowledge about the whole environment and memory of the experiences in it and (2) it can be expressed through novel behavior rather than only through previously rewarded behaviors [1]. Therefore, Tolman also brought a paradigmatic shift to behavioral theory by showing that learning can be more than simply stimulus response associations. It was a second contrasting viewpoint that there might be many types of learning important for survival. 65 years later we are still investigating how these types of learning are processed by the brain.

Place learning could be useful in an evolutionary sense because if an animal was searching for food it once previously cached but was coming from a different location, a place strategy would be necessary to find that food location. Tolman also recognized that place learning required far fewer trials than response learning. A double dissociation experiment by Packard and McGaugh decades later integrated these two theories finding that not only do rats utilize place learning early and response learning later, but that the hippocampus is necessary for the former and the basal ganglia is necessary for the latter (see figure 1.1B) [6].
Chapter 1

Larry Squire model of divisions of memory

Declarative (Explicit)
- Facts
- Events

Nondeclarative (Implicit)
- Skills and Habits
- Priming
- Basic Associative Learning
- Nonassociative Learning
- Emotional responses
- Skeletal musculature

HIPPOCAMPUS, medial temporal lobe
Basal ganglia, motor cortex, CEREBELLUM
Neocortex
Amygdala
CEREBELLUM

Figure 1.1B: Squire model of memory.
Memory has been divided into several categories based on brain region. Declarative memory is divided into facts (semantic memory) and events (episodic memory). There are several forms of non-declarative memory. While this schematic is simplified and controversial, it offers a basic structure for scientific investigations of memory. Brain regions (red) and functions (green) discussed in this thesis. Adapted from: Squire et al., 2012.

1.2 Declarative and procedural memory

1.2.1: HM and declarative memory

Henry Gustav Molaison, also known as H.M. to the scientific community, was a man who had severe epilepsy. It was so severe that by the time he was 27, just 5 years after Tolman’s seminal paper “Cognitive Maps in Rats and Men”, HM was referred to a neurosurgeon by the name of Henry Scoville in 1953, who bilaterally (both sides of the brain) removed his medial temporal lobes, including his hippocampal formation and parts of adjacent structures such as the amygdaloid complex and entorhinal cortex [7]. After the surgery, although the debilitating seizures discontinued, he could no longer make new memories of explicit events; he suffered from severe anterograde memory loss. However, his short-term memory and procedural memory were still in tact. Procedural memory is memory for skills and behaviors. So even though he would see the same doctors every day, he would not remember who they were. He would do tests such as tracing a line on a piece of paper in front of a mirror (difficult procedural memory task) and become very good at it over time, but never remember the event of practicing such a task. This offered a clear distinction between qualitatively different forms of memory that linked anatomical substrates.
The case study of HM, while horrific to the individual, sheds light on many aspects of learning and memory and its relation to the temporal lobe. We learned that episodic and procedural memory were mediated by distinct regions of the brain and that the hippocampus was not just part of the emotional circuit [7, 8]. A plethora of both anatomical and functional discoveries were made from this one case study, and from here experimental designs exploded with the aim to further unravel how learning and explicit memory are related to the temporal lobes [9].

1.2.2: Cerebellar patients and procedural memory

Classical conditioning is commonly thought of as a type of procedural memory and it is typically used as a method to investigate cerebellar mechanisms in learning in animals [10-13]. In humans, signs of cerebellar damage include ataxia, or the lack of voluntary coordination of muscle movements, loss of balance, smooth coordinated movements and an unsteady gait [14]. However, cognitive effects of damage to the human cerebellum have been far from clear. In fact awareness of the contingencies in human classical conditioning typically described as procedural learning, affect the acquisition of classically conditioned responses [15]. One study investigating eye-blink conditioning acquisition in humans with cerebellar damage found severe impairment of conditioned response acquisition. This study and others led to the authors’ conclusion that cerebellar lesions only compromise forms of non-declarative learning that involved the modification of motor responses and that cerebellar lesions have no effect on perceptual or cognitive skill acquisition [16] such as acquisition of conditional discrimination [17, 18]. More recently there seems to be evidence both for and against these assertions (Galliano et al., 2013)[19]. The function of the human cerebellum is further complicated in diagnosis of cerebellar disease such as spinocerebellar ataxias (SCAs). SCAs are an inherited progressive ataxia disease that are very heterogenous and includes various genetic mutations. Some of these phenotypes show purely cerebellar ataxia, while others include symptoms such as visual loss, epilepsy and mental retardation [20]. While lesion and disease exhibit differently when considering neural function, human and animal research is developing evidence that the cerebellum is important for more than non-declarative learning that involves the modification of motor responses.

For example, in contrast to declarative memory which will be discussed in more detail below, procedural memory is thought to involve several brain regions including the motor and premotor cortex, the striatum and the cerebellum. The motor cortex is thought to direct the flow of muscle contractions while the premotor cortex plays a central role in the preparation of movement and sequencing of motor coordination over time. However, these cortical structures work through the subcortical structures of the striatum and cerebellum. The striatal pathways are associated with the globus pallidus and receive cortical input from the entire cerebral cortex and send outputs to the brainstem [21]. However there are minimal
projections to the brain stem nuclei and none to the spinal cord from the globus pallidus. The striatum has been suggested to have a role in higher order motor functions such as the planning and execution of complex motor sequences and the planning and execution of goal-oriented behavior.

The cerebellum, is connected to the rest of the brain by three peduncles, or stalks, the superior, middle and inferior cerebellar peduncles. The middle peduncle receives afferents from the contralateral pontine nuclei while the inferior peduncle receives afferents from the brain stem and spinal cord. Efferents toward the brainstem, thalamus, and hypothalamus arise from the superior peduncle while efferents to the vestibular nuclei arise from the inferior peduncle. Therefore, the cerebellum receives somatic sensory input directly from the spinal cord and has bidirectional connections to brain stem nuclei associated with spinal cord function. It stands to reason that the cerebellum helps to execute fine motor movements, acquire conditioned reflexes and body stability. Although solid evidence has been acquired to support the role of the cerebellum in classical conditioning paradigms and those involving reflexive behaviors, such as eyeblink conditioning and vestibulo-ocular reflex response and adaptation, evidence is surfacing for other roles of the cerebellum in procedural learning and cognitive processing [19, 21, 22]. These new roles for the cerebellum seem to integrate well with neuroanatomy since there are bidirectional connections involving cerebellum and cortical areas as well as connections to brainstem and spinal cord areas [23]. Clinical studies have shown patients with cerebellar damage do not only show motor deficits but also cognitive deficits not associated with motor abnormalities [24].

Although declarative and procedural memory have historically been discussed as separate types of memory that also require distinct neural regions, there is recent evidence for a shared resource between the two [25, 26]. This resource is related to the time scales necessary for adaptive systems. Tasks such as saccade and reaching adaptation are characterized by a fast process, which learns from sensory prediction error but also decays quickly. A slow adaptive process that supports motor memory is also evident that shows little decay over time. Rapid learning and fast forgetting are also properties of the declarative memory system and this shared property is probably not coincidental. Indeed evidence that fast motor memory is disrupted by a declarative memory task, but slow motor memory is not, supports the claim that these two processes (slow and fast motor memory) have distinct neural bases and the non-declarative fast process learning such as arm and eye movement adaptation share critical resources with the declarative memory system [25, 27, 28]. Evidence points to more than one site of plasticity for cerebellar dependent learning where the fast process is associated with the cerebellar cortex and the slow process is associated with the cerebellar nuclei [29, 30]. It has been suggested that the early part of training in a motor task involves higher level attentional and working memory whereas the latter part of motor adaptation does not. Therefore the cerebello-prefrontal network may form the neuronal basis of the fast but not the
slow process for generating motor memory [28]. If this were true it would explain the results cited above, that is, a competition for resources during fast motor learning and declarative learning.

While there is a plethora of evidence showing the role of the cerebellum in the timing of motor control [26, 31, 32], questions about multiple timing mechanisms in the brain have been raised [33]. Multiple timing mechanisms have been recognized as a result of data showing that damage to a wide range of neural systems can cause disruptions on tasks involving temporal information processing. However these data may be a result of cognitive tasks that require the integration of a number of operations one of which could be the representation of time. Furthermore, there is evidence that not only timing of actions, but the perception of timing is affected in cerebellar patients, but not cortical patients in a finger tapping and auditory time-perception task [26]. There are several regions of the cerebellum that project to cortical structures as well as to spinal structures [34-36]. Some anatomical connectivity in humans points to medial cerebellum as important for motor implementation and lateral cerebellum important for timing [26].

1.2.3: Plasticity

While Pavlov, Skinner and Tolman were investigating behavior in the 1920s, 30s and 40s, Santiago Ramon y Cajal was investigating the brain. He is considered the father of neuroscience and his major contributions to neuroanatomy and functional theory allowed for a platform from which neuroscience could build. His detailed drawings of structures such as the hippocampus, and cerebellar purkinje cells (see figure 1.2A) were monumental undertakings considering the time and much of his work is still relevant today. Furthermore, he provided evidence for the neuron doctrine which among other things stated that neurons were individual neural units with specialized features including dendrites, the cell body, and an axon, which use synapses for communication among each other, neural machinery necessary to understand in neuroscience. This doctrine was an observation of historical work, true to the collaborative nature of science of which Ramon y Cajal developed some of the major conceptual framework [37].

![Figure 1.2A: Drawings by Ramon y Cajal.](image)

*Drawings of rodent hippocampus (left) and cerebellar purkinje cells and granule cells from the pigeon (right) made by Ramon y Cajal. He used Golgi’s silver nitrate staining to visualize such structures for drawing. (Left: Nerveux de l’Homme et des Vertabrates, vols 1 and 2 1911)(Right: 1899: Instituto Santiago Ramon y Cajal, Madrid, Spain) Public Domain.*
While Tolman, Pavlov, Skinner and others were making headway in understanding animal behavior and learning, Cajal laid the framework for Donald Hebb who in 1949 gave us insight into a neural mechanism that may facilitate such learning.

Donald Hebb synthesized much of the current research in 1949 and postulated “When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased”. The axon which emanates from one neuron delivers an electrical impulse to neighboring neurons and if the chief neuron (neuron A) excites the neighboring neurons enough, it will cause that neuron to fire. As this process occurs more often, the ability for the chief neuron to cause the neighboring neuron to fire is increased, and it becomes more efficient [38]. This theory was further solidified by Eric Kandel in the sea snail. Using a model organism with very few neurons, Kandel was able to use a reductionist approach and plainly show not only an example of Hebbian learning but the molecular mechanisms underlying it [39-41].

These figureheads and their history have been described because they illustrate several important aspects to this thesis. HM showed that the temporal lobe assists declarative memory whereas it does not for procedural memory. Pavlov, Skinner, and Tolman advanced theories important for an understanding of behavior and conditioning and therefore laid the groundwork for neuroscientific inquiry. Hebb and Kandel postulated a theory for understanding how neurons behave during learning and indirectly how the brain as a whole is plastic or responsive to its outside environment. These people aided paradigmatic changes in the way scientists do research and challenged the popular view of the time.

1.3 The hippocampus and declarative memory

1.3.1: Why the hippocampus?

As has been intimated above learning and memory are important biological functions. The hippocampus is crucial for explicit memory. We used a translational behavioral paradigm to address specific questions about the role of the hippocampus in declarative memory. We hypothesized that (1) recognition of events was made up of two components, familiarity and recognition, and that these components are supported by distinct brain regions (2) that the hippocampus does not support spatial memory only and (3) hippocampal firing patterns driven by local non-spatial cues, will predict the future trajectory of the animal during a conditional discrimination task. In the present thesis we will present evidence for hypothesis one and two and against three.
1.3.2: Hippocampal structure and function

The hippocampus is part of the medial temporal lobe system, one of many systems for memory. It is important for working memory and declarative memory. Declarative memory can be further categorized into episodic memory and semantic memory (See figure 1.1B). Episodic memory is the memory of events that occur in time, such as a conversation at a cocktail party, whereas semantic memory is characterized by the learning of facts, such as the date of the bombing of Rotterdam in World War II (unless of course you were alive at that time and experienced the aftermath). You might not remember when you learned that fact, or what events occurred when you learned it, but over time it has become a fact available for retrieval in your memory. Episodic memory and semantic memory are two forms of cognitive memory as distinguished from behavioral memory. Cognitive memory is flexible and inferential and it allows for the capacity to generalize across many experiences, whereas behavioral or procedural memory (discussed in chapter 3) does not require directed attention. For this reason, cognitive memory and behavioral memory are also known as explicit memory in the former, and implicit memory in the latter.

This functional organization for remembering an event begins at sensory neocortical areas and converges onto the hippocampus. Two streams of information in the brain occur which can be functionally and anatomically distinguished, the dorsal pathway, via the posterior parietal cortex for remembering “where”, and the ventral pathway via the inferior temporal cortex for remembering “what” (see figure 1.3A). These pathways take information from the sensory cortices, combine crucial information in association areas, and continue independently in the parahippocampal region, a region surrounding the hippocampus.

It is contended that the “what” pathway continues via the perirhinal cortex and lateral entorhinal cortex for encoding object features, whereas the parahippocampal and medial entorhinal cortices contain the “where” information representing the experienced context and location of the objects. These two streams converge at the hippocampus to represent items-in-context, and there is evidence that back projections to parahippocampal-medial entorhinal cortices are important for recall of context whereas back projections to the parahippocampal-lateral entorhinal cortices are important for the recall of item associations [42, 43].

Take for example the common experience of remembering someone’s name. You may see someone and not remember their name, but you do remember their face. Their face may represent one item that is processed through the “what pathway”, but maybe you don’t remember where you met them (context) processed through the wher pathway. Since you also cannot remember their name (another object feature) the hippocampus has too little pieces of information to unitize the event. This would be an example of familiarity, it occurs rapidly and varies from a weak sense of knowing something, to a strong belief.
Figure 1.3A: Functional Model of Declarative Memory.

A functional model of declarative memory, inputs and outputs as described by Eichenbaum and Cohen (1985). Two parallel pathways from cerebral cortex make their way to the hippocampus (lower gray box) where items and context of an event are unified. Pink signal indicates theta rhythm from entorhinal cortex to hippocampus important for encoding and retrieval. (DG) dentate gyrus, (SUB) subiculum. Adapted from Lee and Lee 2013.

Now, the person you are speaking with, mentions something about baseball, and this provides you with context (the where pathway) which triggers the memory of the event you met them, and their name (item-in-context). This is referred to as recollection. Recollection, occurs when qualitative associations are brought about through a critical cue. There is evidence that in humans, familiarity and recollection are two different types of declarative memory that show different functional characteristics [42, 44]. The case study of HM exhibited an inability for recognition memory from experiences after the surgery, when his whole hippocampus was removed bilaterally as mentioned above. However, one question that has remained is whether or not these memories are graded memories of the same type supported by the hippocampus or are supported by distinct brain mechanisms and/or distinct brain regions [44], but see also [45] and[46]. Our aim in chapter 2.1 was to investigate the question above.

The hippocampus itself is part of larger functional system called the hippocampal formation. Sub-regions of the hippocampus proper include regions CA3, CA2, and CA1, while the hippocampal formation includes the dentate gyrus (DG), subiculum, presubiculum, parasubiculum and entorhinal cortex (EC). The hippocampal formation consists of a set of unidirectional excitatory pathways. The majority of input to the hippocampus comes from the entorhinal cortex.
Early discoveries of the anatomy of the hippocampus described the trisynaptic circuit which included unidirectional connections from EC>>DG>>CA3>>CA1[47]. These three synaptic projections are termed the perforant pathway, the mossy fiber pathway, and the Schaffer collaterals (see figure 1.3B). These pathways play a wide role in, among others, memory for encoding, retrieval and consolidation but are beyond the scope of this thesis.

1.3.3: Hippocampal Place Cells

One of the contributions the hippocampus makes is in processing spatial memory, a type of declarative memory. Hippocampal place cells were first described in a seminal paper in 1971 by O’Keefe and Dostrovsky. These cells increase their firing when the animal is in a certain place in the environment termed the cell’s place field [48]. They initially found place cells in the CA1 pyramidal layer of dorsal hippocampus and later found they were represented in ventral CA1 as well as the CA3 subfield of hippocampus and dentate gyrus. At the time, there was much speculation in the field and many investigators were still concerned with what these cells are actually representing. James Ranck, a prominent scientist in the field speaks of an experiment he did before he was aware of O’Keefe and Dostrovsky’s work. He described a neuron that “fired when the rat sniffed at the empty hole of the removed water spout in the arena. This cell also fired if the rat simply stood near the empty water-spout hole, if he licked the floor under the water hole…” The behaviorist notion of stimulus-response and the power of reward was still engrained in many. He states emphatically,

“Evidently I saw place cells, but did not understand what they were. One cannot see something unless one knows what to look for. As Pasteur said, ‘chance favors the prepared mind.’ Okeefe’s mind was prepared; mine was not.”
Ranck later went on to work with O’keefe and Nadel and was exposed to cognitive psychology for the first time. It was during this time he was first exposed to Tolman’s cognitive map theory [49] pointing to the increasing relevance of diverse perspectives from which to view neuroscience.

Those that challenged O’Keefe’s theory of place fields and their link to a cognitive map, asked important questions. Are the place cells something that the rat does, or are they related to the environment? If they are related to the environment is it a stimulus in the environment (a reward?) or some other information such as the place itself? If it is in fact the place in the environment that the cell is related to, how does it identify such a place? Does the cell use several cues or only one, a certain type of cue, or any cue? In relation to the environment, will these cells map onto one environment or many and if it is many, is there a relationship to the mapping of one cell onto many environments? Following the discovery of place cells, O’Keefe and Conway set out to answer many of these questions [50].

The 1978 study revealed that a large percentage of place fields could represent more than one environment and that place fields do not only represent space in a rewarded navigational task. They also found that there are more place fields on the perimeter of the platform than toward the center, which indicated that the rim of the platform may act as a cue for the place units. Furthermore, there was no readily apparent topographic relationship between the place fields’ that were represented in both environments. In that regard, this experiment did not reveal how place fields develop. However, this discovery paved the way for an explosion of hippocampal research and questions to be answered. Spatial processing soon started to dominate the hippocampal literature. In parallel, others began to build from this landmark study to determine what inputs into the hippocampus allowed for such phenomenal computational representations.

1.3.4: Head-direction cells and Grid cells

By 1990 there was still much deliberation of the role of place cells in memory and the role of the hippocampus. Studies in this area continued on a systems level approach, starting to shed light on what inputs play a role. Taube and colleagues (1990) reported a cell in the postsubiculum with discriminating spatial qualities that would begin to hint at another component for a possible network for spatial navigation. Head direction cells are defined as a cell that discharges as a function of the animal’s egocentric head direction in the horizontal plane, independent of the animal’s behavior, location or trunk position [51, 52]. Each head direction cell had a preferred direction and its firing rate depended only on the angle between the midline of the head in reference to its position in the chamber. Furthermore the preferred direction was constant over the entirety of the environmental space and also environment independent. This brain region and its anatomical proximity to CA1 of hippocampus had obvious relevance for those studying place cells.
Fifteen years after Taube’s seminal paper on head-direction cells, Hafting and colleagues from the Moser group discovered spatial representations in dorsocaudal medial entorhinal cortex. The authors described grid cells as the basis for a directionally oriented, topographically organized neural map of the spatial environment. This unit fires when the animal’s position coincides with the vertex of a predetermined grid of equalateral triangles spanning across the floor of the entire environment [53]. In other words, in contrast to a place cell which fires in one particular place as an animal traverses an open field, a grid cell will fire in several locations of the open field. If you superimpose when the cell fires onto the location of the animal, the locations in which a grid cell fires is equally distributed, forming small equilateral triangles across the open field (see figure 1.3C). This study characterized many aspects of the nature of grid cells in one study. It proposed the idea that context independent position is information processed upstream of the hippocampus, a function that had originally been thought to be computed in hippocampus.

The study also found, like place cells, the grids were driven by external cues in the environment. However, the grids remained, even when the rats were placed in complete darkness removing visual input. These findings suggest that the existence and maintenance of the cell’s firing properties are not tied to allothetic cues and is possibly a hard-wired representation. This proposal is substantiated by the grid pattern expression at the outset of exposure to a novel environment.

In summary, the postsubiculum, where head direction cells were first located, sends projections to deep and superficial layers of medial entorhinal cortex where grid cells are located. Layer two and three of entorhinal cortex sends projections to the dentate gyrus, CA3 and CA1 subfields of hippocampus all of which show evidence of place cells. The hippocampus sends projections to the postsubiculum both direct and indirect via the subiculum creating a functional loop for ego- and allocentric spatial representations for navigation [54].

1.3.5: Trajectory dependent cells in hippocampus

However, as stated, it was also found that cells in hippocampus sometimes represented even more information than simply the space the organism occupied. Context dependent place fields included cells that only fired when there is an expectation of a future goal location,
recent or upcoming behavioral sequences or trajectories and differential cognitive strategies that could be either spatial or non-spatial [55-57].

For example, in a continuous alternation task, where a rat runs down the stem of a T-maze and alternately turns left or right at the intersection of the T, CA1 pyramidal cells fire differentially depending on whether the rat will turn left or right. The animal needs to know where it came from in order to make a correct turn in the next trial to get a reward at the end of the T. There are also cells that fire depending on where the rat came from. These firing patterns are referred to as prospective firing; when the cell fires depending on where it will go, and retrospective firing; depending on where it came from. Collectively, these firing patterns in hippocampus are known as context-dependent firing. Keep in mind that these firing patterns are based on firing rate, and not topographically organized in any particular brain subregion.

These studies not only questioned the exclusivity of space representation in place field phenomenon, but questioned whether modality specific (only visual) cues were required for the rise of trajectory dependent coding in hippocampus. It also further raised the question as to whether or not trajectory dependent coding in hippocampus was necessary for hippocampal dependent spatial discrimination tasks. Interestingly, the continuous alternation task, where the rat continuously runs in figure 8 patterns, does not require an intact hippocampus, but in a delayed alternation task, where there is a delay at the start of the stem, hippocampus is required. Trajectory dependent firing does not occur while moving along the stem of the T-maze in this task, but does during the delay [58, 59].

Therefore, in chapter 2.2 we aimed to investigate whether or not trajectory dependent coding required spatial cues only or could arise from multi-sensory cues devoid of the spatial parametric space. That is, we asked whether trajectory dependent firing necessitated a cue with a spatial component (where the cue relates to the place it came from), or if the neural response was based on the response (when the cue is based on multiple senses and not a place). We found that fewer cells fired depending on the future trajectory of the animal on a T-maze when the cue was visual-tactile and the trials were discrete whereas in a continuous alternation task more cells fired based upon the future trajectory.

Although it may seem that episodic memory and spatial navigation are considered two separate functions of the hippocampus, that is not the intention of these chapters. Spatial navigation may be considered a subcategory of memory in general, and only separated by topic for simplicity. Over the course of evolution, cortical, limbic, and subcortical areas became more interconnected, perhaps gaining specificity of function as well. As animal experimentalists we keep this in mind in an effort to find fundamental mechanisms as well as region specific. The hippocampus is phylogenetically old, but less so then the cerebellum, also important for learning. Valentino Braitenburg warns “In defense of the cerebellum”
that the fact that this region extended in the anteroposterior direction 10 times its width (during evolution), suggests that it is not just surface area that counts in the cerebellum, but connections within the x and y dimensions [60].

1.4 The Cerebellum, procedural memory and the whisker system

1.4.1: Why the cerebellum and why the whiskers?

The cerebellum is important for sensorimotor integration and procedural learning. Many animal models used to investigate the cerebellum focus on reflexive behaviors, that is classical conditioning that pairs a stimulus with a reflexive response. For example pairing a tone to a puff to the eye, which subsequently and reflexively closes (eye blink conditioning) is an example of classical conditioning. However, cerebello-cortico interactions are also relevant to acquisition of skills and habits and the integration of sensory input and motor output. The whisker system is an ideal model for studying cerebellar processing in that the sensory and motor connections are well known from whisker to cortex, and the behavior can be quantified precisely. We aimed to (1) characterize how the cerebellum encodes whisker input and hypothesized that (2) blocking inhibitory input to purkinje cells would increase the tonic level of post-climbing fiber pause, simple spike suppression and (3) cerebellar plasticity is important for a whisker based associative conditioning task that requires the neocortex. We found that (1) cerebellar purkinje cells show differential coding characteristics to whisker input depending on direct cerebellar afferents (2) and that blocking inhibitory input does affect post-climbing fiber pause activity, but in the opposite direction and that (3) cerebellar plasticity facilitates learning in a whisker-based associative, non-reflexive localization task.

1.4.2: Cerebellar structure

The cerebellum, lies posterior to the cortical hemispheres and dorsally to the pons straddling the fourth ventricle. It is the only structure in the central nervous system that spans the midline continuously without interruption. There are two main pathways to the cerebellar cortex, one via the inferior olive (IO) and the other via the pontine nuclei while the deeper structure of the cerebellar nuclei receive collaterals from the climbing and mossy fibers (see figure 1.4A). Its main known function is the coordination of motor activities to provide finely tuned motor control, smoothness of motion and motor learning [61]; [62].

One of the most extraordinary features of the cerebellum is its enormous structural convergence and divergence. Although it is estimated the rodent cerebellum only occupies 9% of total brain volume, in rats it contains ten billion of the 12 billion neurons in the brain owed in large part to the granule cells [63]. The axons of granule cells emanate dorsally and then transversally to the cerebellar cortex creating mossy and parallel fibers respectively.
Granule cell parallel fiber outputs converge onto purkinje cells in the intermediate layer and the purkinje cell layer converges onto cerebellar nuclei cells and is estimated at 20-50 purkinje cells per one cerebellar nuclei cell [64]. In addition to the synapses that several parallel fibers make onto a purkinje cell dendritic tree, the climbing fiber, emanating from the inferior olive, also synapses onto the dendritic tree of the purkinje cell giving it direct impact on its firing [65]. The climbing fiber input causes characteristic action potentials called complex spikes (CS) while the parallel fiber input modulates simple spikes (SS) from the same cell. Therefore the purkinje cell fires two different kinds of spikes (or action potentials) depending on which input is activated [66] (See Figure 1.4B).

![Figure 1.4B: Purkinje cell action potentials.](image)

Characteristic purkinje cell complex spikes and simple spikes

Characteristic climbing fiber pause

The cerebellum can be longitudinally delineated via regions running along the rostral-caudal axis. The vermis (medial), the paravermis (pars intermedia) and the hemispheres (lateral cerebellum) are folded into lobules and further divided into folia. However the cerebellum is often described depending on experimental bases. The longitudinal zones are divided by observing anatomical and physiological properties with specific olivocerebellar and corticonuclear connections. Further physiological distinctions include microzones which are subdivisions of purkinje cell longitudinal zones that have similar climbing fiber receptive fields [61, 67].

These microzones can be further divided into patches where regions of the cerebellar cortex have similar mossy fiber receptive fields in the granular layer. Further anatomical denotations often include modules which include a longitudinal zone of purkinje cells with olivo-cortico-nuclear connections and their corresponding recurrent pathways. Finally, gene expression patterns occur in the transverse plane where purkinje cell stripes and bands exhibit the same phenotype [61].
1.4.3: Purkinje cell inputs and cerebellar function

One feature of IO is that the cells of this region are connected via gap junctions, a substrate for electrical synaptic connections rather than electrochemical synaptic connections via neurotransmitters. These electrical connections allow for fast communication between neurons, which has been termed electrical coupling.

Lesions of the cerebellar nuclei which receives afferents from the cerebellar cortex and is upstream of the IO showed purkinje cell CS activity as highly synchronous but also non-rhythmic, suggesting the cerebellar nuclei (CN) may be more important for coordinating such CS synchrony while oscillations in IO orchestrate the overall timing [68]. Purkinje cell recordings in crus 2 show parasagitally-oriented CS synchrony while mutants lacking gap junctions have synchrony at chance levels with no preferential spatial orientations. These mutants also exhibited lower CS firing rates indicating electrical coupling is important for IO excitability [69]. Furthermore, deficits in locomotion and eye-blink responses demonstrated a role for electronic coupling in learning dependent timing in cerebellar motor control [70]. Therefore, one question we asked was whether complex spike synchrony would be induced during whisker stimulation, and if so to what extent. This question was answered in Chapter 3.1.

Although complex spike activity arising from the inferior olive shows spatial selectivity following sensorimotor activation due to the microzonal structure converging at the level of the purkinje cell, molecular layer inhibitory interneurons may also be a spatially selective contributor to cerebellar output. Basket cell axons extend along the purkinje cell layer perpendicular to the direction of the parallel fibers. It is estimated that they may contact as many as 150 purkinje cell bodies and as many as 50 different basket cells wrap their axon terminals around each purkinje cell soma, forming a basket-like mesh around the purkinje cell [71]. Stellate cells synapse on the purkinje cell dendrite and also receive input from the parallel fibers. They are found more superficially than basket cells and are larger as you travel deeper into the molecular layer of the cerebellar cortex.

These interneurons have been shown to provide feed-forward inhibition crucial to the purkinje cell, hence cancellation of this input can increase simple spike firing of the purkinje cell but also disrupt not only spatial (via the mesh-like organization described above), but temporal precision of simple spike output [73] but see also [72].

In one study, a mouse mutant was created where the purkinje cell GABA_A receptor γ2 subunit was selectively deleted effectively minimizing feed forward inhibition onto purkinje cells. This study showed an increase in purkinje cell, simple spike firing regularity while the spiking frequency remained the same as controls. In addition, impairments in vestibulocular reflex and consolidation of gain adaptation were disrupted suggesting that feed-forward inhibition aids in cerebellar and vestibular plasticity. [74].
These data suggest Purkinje cell firing can vary depending on its inputs, both direct, indirect and recurrent, as well as from the intrinsic properties of the cell itself. For example, complex spikes are followed by a pause in simple spike activity [75], also known as the climbing fiber pause [76] (See figure 1.4B). The complex spike is an action potential initiated by activation of climbing fiber synapses on AMPA, NMDA, and mGlu receptors which depolarizes the cell’s entire dendritic tree opening voltage-gated calcium channels [77, 78] causing dendritic calcium spikes [79] followed by a sodium-mediated action potential at the Purkinje cell axon hillock [80, 81]. The period of hyperpolarization of the cell may cause the pause in intrinsic simple spike firing after the complex spike but may also be prolonged due to molecular layer interneuron inhibition activated by climbing fibers that also synapse on the molecular layer interneurons [82]. In fact, although the climbing fiber has large acute excitatory action on the Purkinje cell, it has a cumulative suppressive effect on the Purkinje cells. Indeed, IO inactivation results in increased Purkinje cell firing and IO stimulation decreases baseline firing [83, 84].

So, simple spike patterns can be determined by (see figure 1.4A) (1) intrinsic activity of the cell itself, (2) input from the ascending granule axons, the parallel fiber, (3) the climbing fiber, and from (4) the molecular layer interneurons. However, just as climbing fiber input affects simple spike patterns, mossy fiber activity (5) can indirectly modify climbing fiber activity through GABAergic feedback from the cerebellar nuclei to the inferior olive. These interactions result in acute effects of Purkinje cell firing that also may include the climbing fiber pause discussed above, simple spike facilitation occurring directly after the climbing fiber pause, and simple spike suppression which can occur in continuation of the climbing fiber pause or after a period of facilitation (also described in chapter 3.1 figure 2). Each interneuron may be activated by spillover of glutamate from multiple climbing fibers [82, 85]. The functional relevance of cerebellar simple and complex spike interactions in the whisker system is not well understood, yet there is evidence that these interactions may play a role in plasticity of cerebellar outputs [74]. Some of these questions in relation to whisker stimulation effects are also addressed in chapter 3.1.

It has been suggested that synchronized interneuron activity may result in pauses in Purkinje cell firing, and this in turn may cause a release in inhibition on the cerebellar nuclei [86]. There is also evidence that pauses in simple spike activity does not represent additional information about behavioral or sensory events [87]. These single cell effects are important in investigations from a systems level approach. Sequences of mossy fiber activation from cerebral cortex may reach well tuned “beams” of cerebellar cortex (see figure 1.4A) delivering output concerning both motor planning and ongoing movement [88]. For these reasons we hypothesized that mutants lacking molecular layer feed-forward inhibition would show evidence for an increase in CS triggered SS facilitation and a decrease in CS triggered SS suppression. If molecular layer interneurons suppress Purkinje cell firing after a
complex spike, we would expect longer suppression, or stronger suppression. Furthermore, we questioned how reduced inhibitory input affects simple spike periods of activity of silence and if there are differences in these patterns of activity during spontaneous whisking (motor output) and periods of whisker stimulation (sensory input). We first began analysis in relation to SS activity following the climbing-fiber pause. These data are reviewed in chapter 3.2.

1.4.4: Cerebellar Plasticity

In 1969 David Marr published a theory of cerebellar cortical function later to be known as the motor learning theory. He put forward this theory for how the cerebellum learns to perform motor skills with input-output relations that are responsible for learning movements and learning to maintain posture and balance. In this description two assumptions were made; that each olivary cell responds from cerebral instruction for elemental movements and that the purkinje cell can initiate this elemental movement which corresponds to the above described olivary response from cortical movement instruction [89]. This theory purported that the inferior olive, and therefore sequences of purkinje cell complex spikes, provides the cerebellum with information about elemental movements while mossy fiber input provides context for the purkinje cell. As the action is repeated the occurrence of the context alone can cause the next elemental movement, hence establishing a cerebellar role for motor learning [89].

Four years later James Albus proposed his theory of cerebellar function wherein mossy fiber-granule cell-golgi cell input networks enhance the pattern discrimination capacity and learning speed of purkinje cells. The two early models of associative memory and motor learning had similar views on how each cell in the network processes information; predicting that parallel fiber synapses onto purkinje cells mediated by concurrent climbing fiber inputs facilitate motor learning. However, Marr predicted that synaptic strength (potentiation) would be facilitated by learning while Albus believed that these synapses would be weakened (depression) during learning [89].

In order for cerebellar long term depression (LTD) to occur, both the parallel fiber and climbing fiber must be activated simultaneously or in the ideal situation the PF are activated a few hundred milliseconds prior to CF activation [90]. In the context of cerebellar LTD a cascade of molecular events occur that result in an increase in calcium concentration at the post-synaptic cell and eventually a down-regulation of AMPA receptors at the post-synaptic site causing a decrease in efficacy of glutamatergic response, or depression of post-synaptic activation. In contrast to CA1 hippocampal pyramidal cells that require an increase in calcium at the post-synaptic site for long term potentiation (LTP), purkinje cells require an increase in calcium for LTD and a decrease in calcium to exhibit cerebellar LTP. Therefore, cerebellar purkinje cells show inversely related properties in the induction of LTP and LTD in both the calcium requirements and kinase and phosphatase activity upstream required for such effects [91].
Figure 1.4A: The olivocerebellar system.
A functional schematic of the olivocerebellar system highlighting excitatory and inhibitory synapses as well as relevant mutant mice to this thesis. Black arrows represent direction of transmission. (PC) purkinje cell, (IO) inferior olive, (UBC) unipolar brush cell, (MLI) molecular layer interneuron, (GC) granule cell, (MDJ) mesodiencephalic junction.
The cerebellar cortical layers are organized from superficial to deep and include the molecular layer, purkinje cell layer, and granular layer respectively. Since purkinje cells are oriented in parallel to each other, each cell is contacted directly by a single climbing fiber and many parallel fibers, but one climbing fiber can innervate approximately 10 purkinje cells (Sugihara 2005).
Therefore, in deleting the protein phosphotase 2B, the above mentioned molecular cascade is affected by removing the phosphorylation state of AMPA receptors, thereby inhibiting cerebellar potentiation. However, similarly to hippocampus, by manipulating this process, intrinsic plasticity of the cell can also be affected [92].

A mouse mutant line with cre expression under the L7 promotor (purkinje cell specific) lacking PP2B was used by Schonewille et al., 2010, and showed impaired potentiation at the PF-PC post-synaptic site [93]. They found that a lack in potentiation causes deficits in vestibulo-ocular reflex adaptation and delayed response in eyeblink conditioning. However, these behavioral paradigms rely on reflexive behaviors. The whisker system utilizes both lower order and higher order brain regions with the cerebellum located centrally in the hierarchy [94].

Therefore, in chapter 3.3 we aimed to investigate the role of cerebellar plasticity in a whisker based object localization task that also requires cortical recruitment of M1 and S1 [95, 96] by training groups of the above mentioned mutant mice to answer several questions. Will reduced potentiation show phenotypes in either whisking behavior, or licking behavior? Is the cerebellum, specifically cerebellar plasticity, necessary for learning and/or performance of a whisker-based object localization task? Will licking response time challenges cause learning deficits in mutant mice?

1.4.5: Whiskers and the whisker system as a model

“Whisking” refers to a rhythmic sweeping action of the vibrissae and an individual cycle is termed a “whisk”. Whisking is an active process that is also adaptive depending on the information that is sought by the animal in a manner best suited for the task [97, 98]. Rats typically display what has been categorized as two types of active whisking, namely, foveal and exploratory whisking, however, to this author’s knowledge these types of whisking have yet to be identified in mice. Exploratory whisking is used to explore the environment and consists of large amplitude and relatively low frequency movements (5-15Hz) while foveal whisking is used to identify objects and textures once interest has been peaked and consists of thrusting the whiskers forward, making smaller movement at higher frequencies (15-25Hz) [99].

However, rats and mice do not always move their whiskers to make discriminations. Although movement vs. stationary states of sensing have been described as active vs passive whisking respectively, recently an alternative description of whisking has been proposed as generative vs. receptive modes of sensing [100] emphasizing the utility of having a stationary sensing device when detecting, for example vibrations of the vibrissae.

While in the generative mode the animal actively seeks contact with the object and palpitates the whisker on the object causing a percept by it’s own motion, in the receptive mode the animal will immobilize their whiskers in order to optimize the collection of signals from
an object that is moving by its own power. So, for example, vibrissae might be used in the generative mode of whisking to approach and distinguish two different objects, such as prey or a rock [101], while the receptive mode would be used to distinguish vibrations incurred on the same object, perhaps to measure movements of the wall of a burrow to detect whether a predator is above. In this instance perception may rely on the blocking of motor output to keep the whiskers immobile [100]. These behavioral modes may be used in order to understand physiological mechanisms at the sensory afferents to illuminate internal representations upstream of regions important for sensory perception.

Tactile encoding in primary sensory afferents may employ an orthogonal triple coding scheme. Locating the (1) vertical coordinates of an object may be encoded by a spatial code. Locating the (2) horizontal coordinates of an object may be encoded by a temporal code and (3) location of the radial distance may be encoded by intensity of neural firing. That is to say, the distribution of activated sensory neurons in the whisker pad is important for coding the vertical coordinates of an object. Importantly, these three codes are mutually independent of each other, but still allow a three dimensional understanding of the animal’s environment. Furthermore, having the same neuron using different codes for each spatial parameter, rather than different neurons with the same code, simplifies pattern separation for sensory perception processed downstream of primary afferents. But these coding schemes will result in very different predictions from every behavioral paradigm. For example, if we hope to know how cells respond to whisker movements or are activated to produce movement in the cerebellum, and we assume these properties are important to the cerebellum, we would expect differential firing patterns from a purkinje cell when discriminating space vs texture if they are already represented differentially at the sensory afferents. However, the behavioral context and methods of inquiry must be held in light of such low level encoding characteristics for processing upstream in cortical and cerebellar areas [102].

For example, the paralemniscal pathway to cortex conveys sensor motion signals (whisking), extralemniscal pathway conveys contact (touch signals), and the lemniscal pathway conveys combined whisker-touch signals. This functional segregation indicates that different sensory-motor processes such as those related to motor control, object localization, and object identification are implemented along different motor-sensory-motor loops [103].

However the paralemniscal pathway does not relay inputs triggered by passive whisker deflection. This pathway operates through disinhibition, and the posterior group of the thalamus forwards sensory information contingent on motor instruction to the cerebral cortex [104]. Furthermore, rodents are blind to the elemental features present in whisker vibration, instead they perceive a composite feature, the speed of whisker motion [105]. And yet they are still able to use an information seeking, cognitive motor strategy instead of a rigid motor programme in texture classification [97].
Therefore the context of the whisker related task, the nature of the whisker mode, and the mechanical manipulation of the whisker itself, should have very different computational necessities at the level of cerebellum, cortex, and therefore within the cerebello-cortico loop.

In the whisker system the inferior olive receives input directly from the trigeminal nuclei which receives its inputs directly from the whisker primary sensory afferents. From the trigeminal nuclei whisker afferents diverge to four major relevant target structures; the thalamus, the facial nucleus, the cerebellum and the inferior olive (IO). The input from the trigeminal nuclei to the IO then diverges again, one contacting contralateral cerebellar cortex via the climbing fibers with excitatory contacts causing complex spikes in purkinje cells and the other excitatory contact directly to the cerebellar nuclei (CN) (See below; section 1.5).

Furthermore, the high prevalence of electrical coupling in IO has led to investigations, and later, evidence that these neuronal connections underlie synchronous activity in the cerebellar cortex. [106];[107];[108]. These discoveries led to further inquiries showing evidence for a role of synchronous cerebellar activity during whisker movements [109]. Therefore the ability of the motor cortex to generate precise whisker movements or perhaps even a sequence of movements, may be modulated by an oscillatory signal arising in the olivocerebellar system. Findings in chapter 3.3 point to a role for cerebellar plasticity in a whisker based discrimination task where general whisking kinematics were shown to be normal, but whisking strategy was not investigated.

One advantage of using the whisker system as a model to investigate cerebellar neural mechanisms is the recent convergence of data from this model. Most literature has focused on disentangling the functional circuits involved at all levels from the sensory receptor to the sensory and motor cortices. In parallel to this line of research, tactile psychophysics of the whisker system has allowed for precise quantification of behavioral capacities during both simple and complex tasks that have already proven region specific dependencies. Therefore, as these two lines of research converge, the model allows for a better understanding from sensory receptor to behavioral choice to investigate functional neural underpinnings of processes such as perception, decision making, sensorimotor integration, and sensorimotor learning [100].
1.5

Anatomical pathways involved in generating and sensing rhythmic whisker movements

The rodent whisker system is widely used as a model system for investigating sensorimotor integration, neural mechanisms of complex cognitive tasks, neural development, and robotics. The whisker pathways to the barrel cortex have received considerable attention. However, many subcortical structures are paramount to the whisker system. They contribute to important processes, like filtering out salient features, integration with other senses, and adaptation of the whisker system to the general behavioral state of the animal. We present here an overview of the brain regions and their connections involved in the whisker system. We do not only describe the anatomy and functional roles of the cerebral cortex, but also those of subcortical structures like the striatum, superior colliculus, cerebellum, pontomedullary reticular formation, zona incerta, and anterior pretectal nucleus as well as those of level setting systems like the cholinergic, histaminergic, serotonergic, and noradrenergic pathways. We conclude by discussing how these brain regions may affect each other and how they together may control the precise timing of whisker movements and coordinate whisker perception.
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Keywords: vibrissa, follicle–sinus complex, barrel cortex, basal ganglia, cerebellum, sensorimotor integration, rhythmic movements, anatomy

General Introduction

INTRODUCTION

Rodents have highly mobile whiskers, with which they can rapidly locate and discriminate objects in their environment. The rodent whisker system has become a popular model system for brain development, experience-dependent plasticity, perceptual learning, repetitive, timed motor responses, sensorimotor integration, and robotics. Of the many brain regions involved in the whisker system, the trigeminal brainstem, thalamus and primary somatosensory cortex (SI), and to a lesser extent the whisker motor cortex (wMI), have attracted most attention (for reviews see Kleinfeld et al., 1999; Deschênes et al., 2005; Brecht, 2007; Petersen, 2007; Allaway, 2008; Diamond et al., 2009). Other brain regions and the structures of the whisker pad itself have received less attention. Here we aim to integrate the current knowledge on subcortical structures into the well-known whisker pathways, thus presenting an overview of the most important structures of the whisker system and their interconnections as a whole. In addition, we discuss how these structures may cooperate to generate and sense whisker movements.

Tactile hairs are specialized hairs that, due to the presence of sensitive mechanoreceptors at their follicles, provide accurate somatosensory input. Tactile hairs which grow from a follicle–sinus complex (FSC) are called “vibrissae” or “whiskers.” Almost all mammals, except humans and egg-laying mammals (monotremes), have vibrissae (Chernova, 2006; Muchlinski, 2010). Vibrissae can grow from all body parts, but are mainly located on the face (Sarko et al., 2011). Most likely, all vibrissae can be moved, but there is a large variability in movement mechanics. Some vibrissae, like the genal vibrissae in the hamster, lack musculature and are moved solely by vascular and connective tissue dynamics (Winek, 1983). Other vibrissae can be moved by muscles involved in the retraction of hairs (m. arrector pili; Hyvärinen et al., 2009), while mystacial vibrissae can be moved by a group of specialized muscles (Brecht et al., 1997; Haidarliu et al., 2010; Sarko et al., 2011). In some species, including shrews (Munz et al., 2010) and rodents such as rats, mice, gerbils, hamsters, chinchillas, and porcupines (Woolsey et al., 1975), the mystacial vibrissae can move fast and rhythmically (Figure 1A). This behavior is called “whisking,” and in accordance we reserve the term “whiskers” here for those vibrissae that can be whisked. Whisking behavior is absent in most species, including well-studied species like rabbits, cats, and seals (Woolsey et al., 1975; Dehnhardt and Kaminski, 1995). The main function of vibrissae is to complement or replace near-vision (Welker, 1964; Gogan et al., 1981; Ahl, 1986). In addition, marine mammals use their vibrissae for long-distance sensing. For instance, a seal may “feel” prey fish at more than 180 m distance (Dehnhardt et al., 2001). Vibrissae also help to locate, identify, and capture prey (Anjum et al., 2006; Munz et al., 2010; Favaro et al., 2011). In addition, vibrissae inform about body posture, especially in water (Ahl, 1982), and play a central role in social behavior (Miller, 1975; Blanchard et al., 1977).

Well-timed, rhythmic whisker movements are instrumental in exploring the environment (Carvell and Simons, 1990; Grant et al., 2009; Hartmann, 2011). When doing so, rats make large whisker...
movements at a relatively low frequency (5–15 Hz). Once their interest has been caught, they can thrust their whiskers forward and make smaller movements at higher frequencies (15–25 Hz) to identify objects and textures (Carvell and Simons, 1995; Harvey et al., 2001; Berg and Kleinfeld, 2003a). Small variations in surface texture may halt the whisker tip for a short while, after which it slips past the fine obstruction (Figure 1B; Nemark et al., 2003; Ritt et al., 2006; Wolfe et al., 2008). Such “slip-stick” movements can trigger stereotypical neuronal responses allowing the animal to sense subtle features of surfaces (Figure 1C; Jadhav et al., 2009). The combination of rhythmic movements and precisely timed sensory input thus greatly increases the acuity of whisker input.

WHISKERS

THE WHISKER PAD

The organization of the whiskers on the mystacial pad varies greatly between different species, but is relatively similar between rats and mice (Woolsey et al., 1975; Brecht et al., 1997). Rats and mice have five rows of whiskers. The upper two rows (A–B) have four whiskers each, while the lower three rows (C–E) each contain about seven whiskers. In addition, there are four particularly large whiskers (“straddlers”), labeled α–β, at the caudal edge of the mystacial pad (Figure 2A). The muscles of the mystacial pad are divided into extrinsic and intrinsic muscles, all of which are innervated by specific branches of the facial nerve (Figure 2A; Dörfl, 1985). The intrinsic muscles are completely situated within the mystacial pad, while the extrinsic muscles have their origins outside the mystacial region (Dörfl, 1982; Jin et al., 2004; Haidarliu et al., 2010). During a normal, exploratory whisking cycle, the whiskers first protract and then retract. Whisker protraction is initiated by contraction of the medial inferior and medial superior parts of the extrinsic muscle m. nasolabialis profundus and completed by contraction of the intrinsic capsular muscles. Subsequent whisker retraction is under control of two extrinsic muscles, the m. nasolabialis and the m. maxillolabialis (Figures 2A,B; Berg and Kleinfeld, 2003a; Hill et al., 2008; Simony et al., 2010). Whisker retraction during foveal whisking is a relatively passive process, involving virtually no muscle activity; during foveal whisking the vibrissae are thrust forward and palpate objects with low-amplitude movements at high frequency (Berg and Kleinfeld, 2003a). Rodents can also move the whole mystacial pad. Pad movements can contribute to the normal whisking cycle (Bermejo et al., 2005), but can also involve rotation or resizing of the whisker pad to optimize object contact (Haidarliu et al., 2010; Towal et al., 2011). For instance, contraction of the m. nasolabialis superficialis moves the A and B rows dorsally, and contraction of m. buccinatorius pars orbicularis oris moves the C–E rows ventrally, thereby adjusting the mystacial field size (Haidarliu et al., 2010). Although the general structure of the mystacial pad is similar in mice (Dörfl, 1982), hamsters (Wineski, 1985), and rats (Haidarliu et al., 2010), minor differences between species do occur, mainly in the organization of the m. nasolabialis profundus (cf Haidarliu et al., 2010).

FOLLICLE–SINUS COMPLEXES

Vibrissae differ from other (pelagic) hairs in that each of their (epidermal) follicles is surrounded by a (dermal) blood sinus, which in most species is composed of a distal ring sinus and a proximal cavernous sinus (Figures 2B,C; Szymonowicz, 1895; Ebara et al., 2002; Kim et al., 2011). It has been suggested that animals can modulate the dynamic range of the vibrissal mechanoreceptors by changing the blood pressure in the blood sinus (Vincent, 1913; Nilsson, 1969; Gottschaldt et al., 1973). In addition, the size of the FSC seems to be adapted for the behavior and environment of the animals. In general, the largest FSCs are found in marine mammals, intermediate FSCs in semi-aquatic species, like otters and water rats, and the smallest FSCs in purely terrestrial mammals (Dehnhardt et al., 1999; Hyvarinen et al., 2009). Larger FSCs make the vibrissal movements more resistant to water, which has a much higher density than air, and allows better thermal insulation of mechanoreceptors to cold or warm water (Dehnhardt et al., 1998, 1999). The larger size of the FSCs of marine mammals is due to the presence of a second, external cavernous sinus (Figure 2C; Sarko et al., 2007; Hyvarinen et al., 2009). In species where the vibrissal system is relatively unimportant, such as marsupials and primates, the FSCs lack a ring sinus (Van Horn, 1976; Hollis and Lyne, 1974; Marotte et al., 1992). Thus, the adaptations in the FSC anatomy are in line with specific behavioral requirements.
The whiskers are organized in rows on the mystacial pad. Mice and rats have five rows of whiskers, as well as four “straddlers” caudal to these rows. Each whisker is associated with an intrinsic capsular muscle [see also (B)]. Extrinsic muscles connect to multiple whiskers. The m. nasolabialis profundus (MNP) consists of two parts, the mediosuperior (PM) and the mediodiagonal (PMI) parts, both of which are involved in whisker protraction. The m. nasolabialis and m. maxillolabialis are involved in whisker retraction. The other extrinsic muscles, including the m. nasolabialis superficialis and the m. buccinatorius, are involved in resizing the entire mystacial pad. The mystacial muscles are almost exclusively innervated by the facial nerve, which leaves the skull via the stylomastoïd foramen (SMF). After leaving the SMF, the facial nerve splits up in two streams. The lower stream consists of the rami buccolabialis superior (RBS) and inferior (RBI), which anastomose in the buccal plexus (BP). From the BP all extrinsic and intrinsic whisker muscles are innervated, with the exception of m. nasolabialis, which is innervated by the upper stream, which includes the rami zygomatico-orbitalis (RZO). (B) Schematic drawing of the follicle-sinus complex (FSC) of the rat. The vibrissa (V) lies within a follicle that is derived from the epidermis and that is surrounded by the glassy membrane (GM). Around the follicle is a blood sinus derived from the dermis, and which is consistent with the prominent role of facial vibrissae during feeding (Reep et al., 2003).
Chapter 1

Transduction of Sensory Input

Trigeminal Nerve

Mechanoreceptors

Vibrissal vibrations are detected by several types of mechanoreceptors with different functional properties. Each FSC is innervated by several small superficial vibrissal nerves (SVN), a single, large deep vibrissal nerve (DVN) containing 100–200 fibers (Rice et al., 1986), as well as a number of unmyelinated fibers at the base of the FSC (Figure 2B). The SVN and the DVN contain mainly Aβ and Aδ fibers. Thickly myelinated Aβ fibers have Merkel cell endings, which are slowly adapting (SA) mechanoreceptors, or lanceolate endings, which are rapidly adapting (RA). Hence, Merkel cell endings will primarily signal ongoing movements, while lanceolate endings will predominantly detect unexpected movements (Gottschaldt et al., 1973; Halata et al., 2010; Lumpkin et al., 2010). Merkel cells are located within the epidermis at two regions: at the rete ridge collar and at the level of the ring sinus (Ebara et al., 2002). Remarkably, in the mystacial FSCs of rats, the Merkel cells at the rete ridge collar are almost exclusively found at the caudal site of the FSC, implying that they predominantly transmit backward deflections (Fundin et al., 1994; Ebara et al., 2002). Circumferentially oriented lanceolate endings are mainly located at the level of the inner conical body, while longitudinally oriented lanceolate endings are mostly restricted to the level of the ring sinus (Ebara et al., 2002). The thinly myelinated Aδ fibers supply a highly heterogeneous group of other endings, including spiny-like, club-like, reticular, spiny, and encapsulated endings. These endings are dispersed through the epidermal sheet of the FSC, but enriched at the level of the cavernous sinus (Ebara et al., 2002; Sarko et al., 2007). The specific functions of these mechanoreceptors are presently unclear. At the base of the FSC are unmyelinated C fibers (Ebara et al., 2002). Since C fibers predominantly conduct nociceptive stimuli, they could signal pulling of the vibrissae.

Trigeminal ganglion

The cell bodies of the trigeminal nerve fibers are located either in the trigeminal ganglion or in the mesencephalic nucleus (see Trigeminal Mesencephalic Nucleus). As a rule, each neuron in the trigeminal ganglion receives input only from a single vibrissa (Kerr and Lysak, 1964; Zucker and Welker, 1969; Lichtenstein et al., 1990). However, neurons receiving input from very small vibrissae may be connected to two or three individual vibrissae (Kerr and Lysak, 1964). In addition, very large deflections of a single vibrissa can cause deformation of the skin, and in that way also activate mechanoreceptors of adjacent FSCs (Simons, 1985). The receptive fields of the trigeminal ganglion are loosely associated in a somatotopic fashion, with the caudal part of the face projecting to the dorsal part of the ganglion, and the rostral part of the face to the ventral part of the ganglion. The whisker projections follow this general pattern (Erzurumlu and Killackey, 1983; Leiser and Moxon, 2006). Originally, it was reported that dorsal vibrissae are represented medially and ventral vibrissae laterally within the trigeminal ganglion (Zucker and Welker, 1969), but Leiser and Moxon (2006) could not reproduce this medio-lateral patterning. During rest, when the vibrissae are neither moving nor being touched, the neurons of the trigeminal ganglion are silent (Gibson and Welker, 1983; Lichtenstein et al., 1990; Leiser and Moxon, 2007). Based on their response pattern to vibrissal movement, the majority of trigeminal ganglion neurons are classified as SA, while the others are RA (Fitzgerald, 1949; Kerr and Lysak, 1964; Lichtenstein et al., 1990; Leiser and Moxon, 2007). During whisking in air, SA neurons fire about three times as often as RA neurons (Leiser and Moxon, 2007). Upon touching an object, both SA and RA neurons increase their firing rate. Both types of neurons reach similar firing rates upon whisker touching (Jones et al., 2004; Leiser and Moxon, 2007). Overall, trigeminal ganglion neurons have a broad range of activation thresholds that vary mainly in amplitude and speed, but also in direction of whisker movement (Arabzadeh et al., 2005; Leiser and Moxon, 2007; Khatri et al., 2009; Gerdjikov et al., 2010). Most trigeminal ganglion neurons receive whisker sensory input via the DVN rather than the SVN, but the information content of both types of fibers seems to be very similar (Waite and Jacquin, 1992).

Trigeminal mesencephalic nucleus

A subset of trigeminal nerve fibers does not have their somata in the trigeminal ganglion, but in the trigeminal mesencephalic nucleus (MeV). Thus, MeV houses primary sensory neurons within the CNS, which makes it a unique structure. MeV neurons mainly innervate muscle spindles in the masticatory and extracranial muscles and are thus involved in proprioception. In addition, several other types of receptors in the dental, oral, and peri-oral domain are innervated by MeV neurons (Lazarov, 2002). Although whisker muscles lack spindles, MeV contains neurons that innervate the mystacial pad and that respond to spontaneous whisker movements (Mameli et al., 2010). MeV projects to, among others, the dorsomedial part of the principal trigeminal nucleus, the pontomedullary reticular formation (RF), and the superior colliculus (Sc; Matesz, 1981; Ndaiye et al., 2000).

Sensory Trigeminal Nuclei

The sensory trigeminal nuclei form the main entrance to the brain for whisker input. The principal trigeminal nucleus (PrV) lies anterior to the spinal trigeminal nucleus (SpV), which consists of an oral (SpVo), an interpolar (SpVi), and a caudal part (SpVc; Figure 3A). Afferent fibers of the trigeminal root bifurcate to form a rostral branch ascending to PrV and a caudal branch descending to SpV (Hayashi, 1980). Of the individual fibers, some target only PrV or SpV, while others bifurcate and innervate both. Afferents to SpV can terminate in all three subregions (Hayashi, 1980). All compartments, except SpVo and the rostral part of SpVi, have barrelettes, discrete groups of neurons that receive input from the same vibrissa and that can be visualized by cytochrome oxidase staining (Figure 3B; Belford and Killackey, 1979; Ma, 1991; Li et al., 1994; Erzurumlu et al., 2010). Neurons in the barrelettes are relatively small and their dendritic trees are confined within the borders of the barrelette (Veinante and Deschênes, 1999). Roughly one-third of the neurons dedicated to whisker input are located between the barrelettes. These interbarrelette cells have widespread dendritic trees and receive input from multiple vibrissae, mainly located within a single row on the mystacial pad (Veinante and Deschênes, 1999). The barrelettes are organized according to an inverted somatotopy, with dorsal whiskers having a ventral representation and rostral whiskers a medial one (Ma, 1991; Erzurumlu...
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General Introduction

FIGURE 3 | The trigeminal nuclei. (A) The sensory trigeminal nuclei consist of two nuclei, oriented along the antero-posterior axis. The principal nucleus (PrV) is located at the anterior end and the spinal nucleus (SpV) at the posterior site. The SpV can be subdivided into an oral (SpVo), interpolar (SpVi), and caudal part (SpVc). The facial vibrissae project to the ventral part of the trigeminal nuclei. In PrV, SpVc, and the caudal part of SpVi, each vibrissa has its own projection field: a barrelette. The orientation of the barrelettes of the facial macro-vibrissae is indicated schematically. (B) Coronal section of a neonatal mouse brain, showing the location of the barrelettes of the facial macro-vibrissae in the ventral part of SpVi. Following cytochrome oxidase staining, barrelettes appear as dark patches. Note the inverted somatotopy: dorsal vibrissae project to ventral barrelettes. The smaller patches dorsal to the barrelettes of the E-row are the receptive fields of the facial micro-vibrissae. The photomicrograph was kindly provided by Dr. R. S. Erzurumlu.

et al., 2010). In addition to the large barrelettes representing the whiskers, smaller barrelettes can be seen that mainly represent the facial micro-vibrissae (Figure 3B). We will restrict ourselves to the description of the neuronal circuitry of the whiskers, rather than that of the other vibrissae.

In PrV, output neurons can be found both within and between barrelettes (Venante and Deschênes, 1999). In SpVi, however, single-whisker neurons project mainly within the trigeminal nuclei, while multi-whisker neurons project to other brain regions (Woolston et al., 1983; Jacquin et al., 1989a,b). The small single-whisker neurons of SpVi are part of an extensive, inter-trigeminal network. GABAergic and glycinergic neurons of SpVc project to SpVi, and GABAergic and glycinergic neurons of SpVi project to PrV (Furuta et al., 2008). In addition, glutamatergic interneurons of SpVc project to both SpV and PrV (Furuta et al., 2008). In this way, SpV can modulate the sensitivity of PrV to whisker inputs (Timofeeva et al., 2005; Furuta et al., 2008; Lee et al., 2008a). This SpV-mediated modulation of PrV in turn is subject to modulation by the somatosensory cortex (Furuta et al., 2010). This allows for central control of the whisker sensitivity. Most likely, this pathway is being used during active whisking, when the whisker-induced output of PrV is suppressed (Lee et al., 2008a). Since there is no strong, direct pathway from wM1 to SpV, this effect is most likely mediated by the whisker area of S1 (wS1). Thus, activity in wM1 activates wS1, which in turn activates the inhibitory projection from SpVi to PrV, reducing the output of PrV (Lee et al., 2008a). This could help the trigeminal nuclei to filter out irrelevant inputs, which may be particularly prominent during movement. Another way to reduce irrelevant input is selective adaptation. PrV responses triggered by weak sensory inputs rapidly desensitize, but are relatively unaffected by repeated strong inputs (Ganmor et al., 2010). Finally, the activity of the sensory trigeminal nuclei can be modulated by several inputs that mainly reflect the general state of alertness, including a cholinergic projection from the pedunculopontine tegmental nuclei (PPTg; Timofeeva et al., 2005; Beak et al., 2010), a serotonergic projection from the raphe nuclei (Lee et al., 2008c) and a noradrenergic projection from the locus coeruleus (Moore and Bloom, 1979). Taken together, the level of detail of the sensory information forwarded to the rest of the brain by the trigeminal nuclei depends on the behavioral state of the animal.

Apart from the contralateral projections to the thalamus described in detail below, there are also contralateral projections from the trigeminal nuclei to the pontine nuclei (see The Pontine Nucleus and the Nucleus Reticularis Tegmenti Pontis), the inferior olive (IO; see Cerebellum and Inferior Olive), the pontomedullary RF (see Pontomedullary Reticular Formation), and the lateral facial nucleus. The trigemino-facial connections originate from all four subnuclei, but mainly from SpVc (Erzurumlu and Killackey, 1979; Pingnaud et al., 1999; Hattox et al., 2002). Since the lateral facial nucleus houses whisker motor neurons (Klein and Rhoades, 1985; Herfst and Brecht, 2008), this connection forms a direct feedback loop (Nguyen and Kleinfeld, 2005). It has been suggested that SpV also receives motor input from wS1,
the information of which might be forwarded to the lateral facial nucleus via the direct connection (Matyas et al., 2010).

THALAMUS AND TRIGEMINO-THALAMO-CORTICAL PATHWAYS

The thalamus is the main gateway to the cerebral cortex. It is composed of several nuclei, two of which are critically involved in the transmission of whisker stimuli to wS1: the ventral posterior medial nucleus (VPM) and the medial posterior nucleus (Pom). There are at least six pathways conveying whisker input from the trigeminal nuclei to the cerebral cortex (Figure 4E). To some extent, these pathways convey different aspects of whisker sensation (Yu et al., 2006). The pathways that make synapses in VPM convey whisker input with short latencies, while those via Pom have considerably longer latencies. VPM receives both single- and multiple-whisker input, Pom only multi-whisker input. An anatomical difference between VPM and Pom is that VPM, in contrast to Pom, contains barreloids, analogous to the barrelettes in the trigeminal nuclei, and the barrels in wS1. The barreloids are prominent in the dorsomedial part of VPM (VPMdm), but fade away toward the ventrolateral part (VPMvl; Van der Loos, 1976; Land et al., 1995; Haidarliu and Ahissar, 2001). As a consequence, VPMdm processes mainly single-whisker input and...
VPVm1 multi-whisker input. Within a barreloid, the neurons are ordered according to their angular preference (Timofeeva et al., 2003).

Of all trigemino-thalamo-cortical pathways, the lemniscal pathway is the only one that predominantly conveys single-whisker input. This disynaptic pathway links the barrelettes of PrV to the barrels of wS1 via the barreloids of VPdm (Erzurumlu et al., 1996; Williams et al., 1994; Veinante and Deschênes, 1999). The main targets are the barrels of layer 4 in wS1, but there are also terminals in layers 5/6 of wS1 (Killackey, 1973; Koralek et al., 1988; Chmielowska et al., 1989; Lu and Lin, 1993; Bureau et al., 2006; Peteanu et al., 2009; Meyer et al., 2010a). The thalamic relay cells in the barreloids of VPdm respond with precisely timed single action potentials to deflections of a single principle whisker at short latencies (4–8 ms;Ito, 1988; Simons and Carvell, 1989; Petreanu et al., 2009;Meyer et al., 2010a). The thalamic relay cells in the VPdm-barreloids of VPMdm (Erzurumlu et al., 2003).

A second pathway synapsing in VPM is the extralemniscal pathway. In contrast to the lemniscal pathway, the extralemniscal pathway passes through VPvm4, where the barreloids are not as distinct as in VPdm. The input of the extralemniscal pathway originates from the multi-whisker interbarrelette cells of the caudal part of SpVm, and the output is targeted to layers 4 and 6 of wS2, as well as the septal columns of wS1 (Pierret et al., 2000).

The third pathway, the paralemniscal pathway, arises from the multi-whisker cells in the rostral part of PrV (Erzurumlu and Killackey, 1986; Peschanski, 1984; Williams et al., 1994; Veinante et al., 2000a), contacts relay cells in Pom and targets wS1, wS2, and wM1. Pom axons terminate mainly throughout layers 5a and 1 of wS1 as well as in layer 4 of the septa (Koralek et al., 1988; Chmielowska et al., 1989; Lu and Lin, 1993; Bureau et al., 2006; Peteanu et al., 2009; Wimmer et al., 2010), where they also provide synaptic input to pyramidal neurons in layers 3 and 5a (Bureau et al., 2006; Peteanu et al., 2009; Meyer et al., 2010a). In addition, Pom terminals are found in wS2 and wM1 (Carrell and Simons, 1987). From Pom, there are also projections to the striatum (Alloway et al., 2006), the perirhinal cortex and the insular cortex (Deschenes et al., 1998). Responses of relay cells in Pom to single-whisker deflections differ from those in VPM: in Pom, the receptive fields are larger, the latencies longer and more variable and the activity is under control of a strong cortical feedback (Diamond et al., 1993b; Ahissar et al., 2000). The variable and relatively long response latencies (19–27 ms) of Pom cells are likely caused by inhibitory inputs from ZI gating peripheral inputs to Pom (Trager and Keller, 2004).

In addition, there are at least three other trigemino-thalamo-cortical pathways. All of these convey multi-whisker information. The first arises from the interbarrelette cells of PrV, projections to Pom and to multi-whisker relay cells in the “heads” of the barreloids at the dorsomedial margin of VPM (VPVm4; Veinante and Deschênes, 1999; Urbain and Deschênes, 2007b). The head barreloid cells send axons to the septal columns of wS1 (Furuta et al., 2009). A second multi-whisker pathway involves projections from SpVm to the thalamic laterodorsal nucleus (LD), which projects mainly to the cingulate and retrosplenial cortex, and only sparsely to wS1 (Bezdudnaya and Keller, 2008). And finally, there is a relatively sparse and poorly characterized pathway originating from multi-whisker neurons in SpVm and projecting to the caudal thalamic regions including the most posterior parts of VPM and Pom (Jacquin and Rhoades, 1996; Veinante et al., 2000a). These thalamic regions receive inputs from different sensory modalities and project to the perihinal cortex, striatum, and amygdala (Groenewegen and Witter, 2004).

Apart from being the relay station between the trigeminal nuclei and the cerebral cortex, the thalamus also contains intra-thalamic projections. As such the reticular nucleus (RT) is involved in several negative feedback loops that modulate the flow of information through trigemino-thalamo-cortical pathways discussed above. RT forms a sheet of GABAergic neurons surrounding the thalamus and it contains a somatotopic body map with a large representation of the whiskers (Shosaku et al., 1984; Guillery and Harting, 2003; Pinault, 2004). Axons of VPM and Pom cells give off collaterals in RT (Crabtree et al., 1998; Lam and Sherman, 2011), while RT in turn provides strong inhibitory input to VPM and Pom (Pinault et al., 1995; Cox et al., 1997; Brecht and Sakmann, 2002b). The VPM-projections from RT cells are whisker-specific: they target the barreloid of their own principle whisker (Desilets-Roy et al., 2002). Since RT neurons adapt stronger to repeated, high-frequency stimulation than VPM neurons, strong whisker stimulation can lead to disinhibition of VPM neurons (Hartings et al., 2003; Gammor et al., 2010). Furthermore, VPM cells can influence activity in Pom through intra-thalamic pathways involving RT (Crabtree et al., 1998). Additional indirect inhibitory feedback loops to Pom involve ZI (see Other Structures Projecting to the Facial Nucleus), which receives both peripheral and cortical-thalamic input and provides a significant portion of GABAergic synaptic terminals in Pom (Barthö et al., 2002; Bokor et al., 2005).

**Primary Somatosensory Cortex (S1)**

The whisker part of S1 (wS1) is of crucial importance for perception and processing of whisker input. For instance, wS1 is required for whisker-based object localization (O’Connor et al., 2010b), gap-crossing (Hutson and Masterton, 1986), and aperture width discrimination (Krupa et al., 2001). Direct stimulation of wS1 in rabbits can substitute for peripheral vibrissa stimulation (Leal-Campanario et al., 2006). This suggests that wS1 can form sensory percepts, but does not differentiate between peripheral and central stimulation (see also Huber et al., 2008). Recent evidence indicates that wS1 also has a previously unanticipated role in motor control of whisker retraction (Matyas et al., 2010).

As all cortical areas, wS1 is composed of layers. Layer 4 is the main input layer, and in mice it is organized in patches (“barrels”) of neurons primarily receiving input from a single whisker (Figure 4B; Woolsey and Van der Loos, 1970). Within a mouse barrel, most neurons are found at the borders, leaving the barrel center relatively empty. In rats, a similar organization is found (Figure 4A), but the barrel diameters are larger (∼400 μm) than in mice (∼280 μm), and the cells are equally distributed within the barrels (Welker and Woolsey, 1974). In mice, a single barrel column contains, distributed over all layers, ∼6,300 neurons (C2 barrel; Lefort et al., 2009), while the rat C2 barrel contains ∼19,000 neurons (Meyer et al., 2010b). The barrels are strictly organized in a somatotopic pattern (Welker, 1971). In between the barrels are the septa, which mainly receive multi-whisker input (Brumberg...
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et al., 1999; Furuta et al., 2009). The septa are larger in rats than in mice (Welker, 1971; Woolsey et al., 1975). Within the class of mammals, rats and mice are quite exceptional in having barrels in wS1. Barrels are only present in some rodents, as well as a few other species (Woolsey et al., 1975; Rice, 1985). In adult rabbits, for instance, barrels cannot be identified. Yet, also rabbits probably have a somatotopic representation of their vibrissae in S1, but the borders between the whisker receptive fields are fuzzier than in animals with barrels (Figure 4C; Woolsey et al., 1975; McMullen et al., 1994).

Throughout wS1, sensory-evoked responses are sparse and near-simultaneous, but the response probabilities are layer- and cell-type-specific (Brecht and Sakmann, 2002a; Brecht et al., 2003; Manns et al., 2004; De Kock et al., 2007). In the barrel columns, spiking responses in excitatory neurons across all layers are largely restricted to deflections of the principle whisker, except for thick-tufted layer 5 pyramidals (Welker, 1971; Simons, 1978; Manns et al., 2004; De Kock et al., 2007). Subthreshold synaptic responses, however, can also be triggered by the movement of several whiskers surrounding the principle whisker (Brecht and Sakmann, 2002a). Sensory-evoked responses in layer 4 cells are brief due to the recruitment of powerful thalamo-cortical feed-forward inhibition (Swadlow, 2002; Gabernet et al., 2005; Sun et al., 2006; Cruikshank et al., 2007). Angular tuning domains have been observed within layers 4 (Bruno et al., 2003) and 2/3 in adult rats (Andermann and Moore, 2006; Kremer et al., 2011). During free whisking, neurons across all layers respond to active touch (Curts and Kleinfeld, 2009; O’Connor et al., 2010b; Crochet et al., 2011) and to slip-stick motion events (Figure 1C; JadHAV et al., 2009). Sensory-evoked activity patterns in wS1 correlate well with psychophysical performance in whisker-dependent tactile discrimination tasks (Krupa et al., 2004; von Heimendahl et al., 2007; Stütgen and Schwarz, 2008; O’Connor et al., 2010b). The activity of wS1 neurons encodes the spatial location of the whiskers over time (Fee et al., 1997; Crochet and Petersen, 2006; De Kock and Sakmann, 2009). This is also true for GABAergic interneurons (Gentet et al., 2010). Such a reference signal is required for decoding horizontal object position (Diamond et al., 2008), for example by neurons in wS1 for which phase in the whisk cycle gates the response to touch (Curts and Kleinfeld, 2009).

In comparison to responses in the barrel columns, those in the septal columns are less whisker-specific. The barrel and septal columns have been proposed to represent two partially segregated circuits that process different aspects of whisker movements (Kim and Ebner, 1999; Shepherd and Svoboda, 2005; Alloway, 2008). However, the segregation between barrels and septa, while prominent in rats, is not so clear in other species, like mice which have only very thin septa (cf Bureau et al., 2006). The microcircuit of wS1 has been extensively characterized, yielding increasingly detailed connectivity schemes (Lübke and Feldmeyer, 2007; Schubert et al., 2007; LeFort et al., 2009; Petreanu et al., 2009). Layer 4 barrel neurons, which are the main recipients of the lemniscal pathway, project to all layers within their own barrel column, but most prominently to other layer 4 cells as well as layer 2/3 pyramidal cells (Kim and Ebner, 1999; Lübke et al., 2006; Petersen and Sakmann, 2008; Schubert et al., 2001; Feldmeyer et al., 2002, 2005; Shepherd and Svoboda, 2005; LeFort et al., 2009). Layer 2/3 pyramidal cells project both within their own barrel column as well as over long distances across barrel columns (Lübke and Feldmeyer, 2007). They contact cells within all layers except layer 4, with a particularly strong connection to other layer 2/3 pyramidal neurons and to thick-tufted layer 5b pyramidal cells (Reyes and Sakmann, 1999; Schubert et al., 2001; LeFort et al., 2009; Petreanu et al., 2009). Layer 5a neurons, which are the main recipients of the paralemniscal pathway, project strongly within their own barrel column to other pyramidal cells across layer 5 (LeFort et al., 2009), and to layer 2 cells distributed across multiple columns and preferentially located above the septa (in rats, but not in mice; Shepherd and Svoboda, 2005; Bureau et al., 2006). Layer 2 neurons receive additional inputs from layer 3 neurons located above barrels (Bureau et al., 2006), providing one of several possible points of convergence for the lemniscal and paralemniscal pathways (Lübke and Feldmeyer, 2007). Inhibitory input to excitatory neurons is derived from cells within the same cortical layer as well as from cells from other cortical layers (Helmstaedter et al., 2009; Kätzel et al., 2011). In addition to the aforementioned intracolumnar connections within wS1, intracortical projections extend throughout much of wS1 and its dysgranular zone (Chapin et al., 1987; Hoellinger et al., 1995; Kim and Ebner, 1999; Aronoff et al., 2010).

The whisker area of S1 forms reciprocal connections with several other cortical areas, including the whisker part of the secondary somatosensory cortex (wS2), wM1, insular cortex, and perirhinal cortex (White and DeAmicis, 1977; Welker et al., 1988; Fabri and Burton, 1991; Cauller et al., 1998; Aronoff et al., 2010). The contralateral wS1 is targeted by callosal projections (Larsen et al., 2007; Petreanu et al., 2007). Axonal projections to wS2 originate from the infragranular and supragranular layers of wS1 and arborize across all layers in wS2 (Welker et al., 1988; Fabri and Burton, 1991; Cauller et al., 1998; Chakrabarti and Alloway, 2006; Aronoff et al., 2010). The wS1 to wM1 projection is somatotopically arranged such that a column in wS1 connects to a column of the same whisker in wM1 (Israeli and Porter, 1995; Hoffer et al., 2003; Ferezou et al., 2007). Layer 2/3 pyramidal cells of wS1 densely innervate layers 5/6 of wM1, while those of layers 5/6 preferentially innervate layers 1 and 2/3 in wM1 (Porter and White, 1983; Miyashita et al., 1994; Aronoff et al., 2010). The majority of connections to wM1 arise from neurons located in septal columns (Grandy et al., 1986; Alloway et al., 2004; Chakrabarti et al., 2008). The reciprocal projection, from wM1 to wS1, innervates mainly layers 5/6 and 1 (Cauller et al., 1998; Veinante and Deschénes, 2003; Matyas et al., 2010).

Cortico-thalamic projections originate in layer 5/6 and target relay cells in VPM and POM, as well as GABAergic neurons in RT (Hoogland et al., 1987; Welker et al., 1988; Chmielowska et al., 1989; Bourassa et al., 1995; Deschénes et al., 1998; Veinante et al., 2000b; Killackey and Sherman, 2003). The projections to VPM originate from layer 6a pyramidal neurons (located below both the barrels and the septa) and target the barrelloid of the corresponding principal whisker as well as those of several whiskers located within the same arc (Hoogland et al., 1987; Bourassa et al., 1995). VPMv1, which is the thalamic relay station for the paralemniscal pathway, receives cortical input from layer 6 pyramidal cells, both from wS1 and from wS2 (Bokor et al., 2008). The heads of
the barreloids in VPM, which participate in a multi-whisker lem- niscal pathway, receive collaterals from layer 6b pyramidal cells projecting to Pom (Bourassa et al., 1995; Deschênes et al., 1998). The relay cells of Pom also receive input from layer 6a pyramidal cells (located below the septa) and layer 5b tall-tufted pyramidal cells (located below both the barrels and the septa), whose axons form large and powerful synapses that can drive Pom neurons (Hosglund et al., 1991; Killackey and Sherman, 2003; Larsen et al., 2007; Groth et al., 2008). These relay cells project to wS2, forming a cortico-thalamo-cortical pathway (Thielsel et al., 2010). Layer 5b neurons also project to ZI (Bourassa et al., 1995; Mitrofanis and Mikuletic, 1999; Veinante et al., 2000b; Barttho et al., 2007), which is involved in state-dependent suppression of whisker sensory responses in Pom (see Zona Incerta). RT cells are innervated by collaterals of cortico-thalamic axons from layer 6 cells, but not layer 5 cells (Bourassa et al., 1995), which strongly activate RT cells and evoke dysynaptic inhibition in thalamo-cortical relay cells (Cruikshank et al., 2010; Lam and Sherman, 2010). Other projections of wS1 include projections from layer 5a pyramidal cells to the striatum (see Basal Ganglia) and from layer 5b pyramidal cells to the anterior pretectal (APT) nucleus (Aronoff et al., 2010), SC (see Superior Colliculus), the red nucleus (see Anterior Pretectal Nucleus), the pontine nuclei (see The Pontine Nucleus and the Nucleus Reticularis Tegmenti Pontis), and the sensory trigeminal nuclei (see Sensory Trigeminal Nuclei).

SECONDARY SOMATOSENSORY CORTEX (S2) S2 contains a highly organized somatotopic representation of the whiskers (wS2) that occupies around 14% of the total area of S2 and that is located in the parietal cortex, lateral to wS1 (Carvell and Simons, 1986; Koralek et al., 1990; Fabri and Burton, 1991; Hoffer et al., 2003; Benison et al., 2007). The whisker receptive fields in wS2 are larger than in wS1; wS2 neurons generally respond equally well to several adjacent whiskers (Welker and Sinha, 1972; Carvell and Simons, 1986; Kweyir-Afful and Keller, 2004). Responses in wS2 to single-whisker deflections are weaker than those in wS1, but they display stronger direction selectivity, while the onset latencies are comparable (Kweyir-Afful and Keller, 2004). The local connections within wS2 are similar to those within wS1. However, in contrast to wS1, the projections from layer 2/3 to layer 5 are stronger than those from layer 4 to layer 3 (Hsoks et al., 2011). Furthermore, the reciprocal connections between layers 5 and 6, which are weak in wS1, are more pronounced in wS2 (Hoogs et al., 2011). Whisker input reaches wS2 via the extralemniscal pathway through VPMvl (Pierret et al., 2000; Bokor et al., 2008), but also via P (Carvell and Simons, 1987; Spratea et al., 1987; Alloway et al., 2000; Thielsel et al., 2010) and from both the barrel and septal columns of wS1 (Kim and Ebner, 1999; Chakrabarti and Alloway, 2006). The connections between wS1 and wS2 are reciprocal (Carvell and Simons, 1987; Aronoff et al., 2010). In addition, there are reciprocal connections between wS2 and wM1 (Porter and White, 1983; Miyashita et al., 1994). There are also projections to the striatum (see Basal Ganglia), the pontine nuclei (see The Pontine Nucleus and the Nucleus Reticularis Tegmenti Pontis), and to several thalamic nuclei, including VPM, Pom, and RT (Liao et al., 2010). wS2 also receives cholinergic input from the nucleus basalis magnocellularis (Deureu.velher and Semba, 2011).

WHISKER MOTOR CONTROL Rhythmic whisker movements increase the acuity of the whisker system (Swed et al., 2003; Knutsen et al., 2006). Whisker movements are generated in the facial nucleus, whose activity is affected by a large number of brain regions. It has been proposed that higher-order areas can initiate movement, but that the rhythmicity of the whiskers is caused by a brainstem central pattern generator (CPG; see Serotonin).

FACIAL NUCLEUS The motor neurons of both the intrinsic and the extrinsic muscles of the whisker pad are located in the lateral facial nucleus (Ashwell, 1982; Klein and Rhoades, 1985; Herbst and Brecht, 2008). Of the lateral facial nucleus neurons that evoke whisker movements, about 80% induce the protraction of a single whisker and about 20% the retraction of multiple whiskers (Herbst and Brecht, 2008). Each intrinsic capsular muscle has about 25–50 motoneurons in the lateral facial nucleus (Klein and Rhoades, 1985). The motor commands are forwarded to the whisker muscles via the facial nerve (Figure 2A). (Dorfl, 1983; Haidarliu et al., 2010). In addition, there is sparse innervation of the extrinsic muscles by the hypoglossal nucleus via the infraorbital branch of the trigeminal nerve (Mameli et al., 2008).

Single motor neurons in the lateral facial nucleus evoke fast, short, and stereotypic whisker movements, whereas single neurons in wM1 evoke slow, small, and long-lasting rhythmic movements (Brecht et al., 2004a; Herbst and Brecht, 2008). This discrepancy makes it unlikely that wM1 directly commands activity of the lateral facial nucleus, despite the possible existence of a sparse monosynaptic projection from wM1 to the contralateral lateral facial nucleus (Grinevich et al., 2005). Instead, wM1 may induce rhythmic whisker movements via oligosynaptic pathways to the lateral facial nucleus. Remarkably, rhythmic whisker movements persist in the absence of wM1 (Welker, 1964; Semba and Komisaruk, 1984; Gao et al., 2003). Hence, it has been proposed that wM1 projects to a CPG in the brainstem, possibly the dorsal raphe nucleus, that in turn activates the lateral facial nucleus (Hattox et al., 2003; see Serotonin). In addition, the lateral facial nucleus receives input from several other subcortical structures, all of which are directly or indirectly innervated by wM1. These afferent regions include the ipsilateral sensory trigeminal nucleus (Nguyen and Kleinfeld, 2005), the ipsilateral pontomedullary RF (Zerar-Maillé et al., 2001), and the contralateral SC (Miyashita and Mori, 1995; Hattox et al., 2002). In addition, the lateral facial nucleus is targeted by cholinergic, histaminergic, and noradrenergic connections, which may set the overall activity level of the whisker movements (see Arousal, Alertness, and Attention). Altogether, there is a strong convergence of inputs at the level of the lateral facial nucleus, allowing the integration of whisker movements and other forms of behavior.

CEREBRAL CORTEX Primary motor cortex (M1) The primary motor cortex (M1) is a large area in the frontal cortex involved in movement. M1 has an agranular appearance, low stimulation thresholds for evoking movements, and a topographic and complete representation of the body muscles.
Microstimulation of wM1 can induce both whisker protraction and retraction depending on the location of stimulation in wM1 (Gioanni and Lamarche, 1985). M1 can be divided into the agranular medial field (AGm), the agranular lateral field (AGl), and the cingulate area (Cg1). The topographic representation of whiskers is almost exclusively located in AGm (Brecht et al., 2004a). Sensory input from the whiskers to whisker M1 (wM1) comes predominantly via wS1 (Armstrong-James and Fox, 1987), but also directly from Pom (Deschenes et al., 1998). The latencies to whisker stimulation are 10–20 ms longer in wM1 than in wS1 (Figure 4D; Ferezou et al., 2007). Microstimulation of wM1 can generate whisker motion that strongly resembles natural exploratory whisking (Berg and Kleinfeld, 2003b; Brecht et al., 2004b; Haiss and Schwarz, 2005; Matyas et al., 2010). During a training paradigm, mice can learn to protract their whiskers following an auditory conditioned stimulus (CS; Troncoso et al., 2004). Such associative learning probably involves synaptic plasticity of layer 5 pyramidal cells in wM1 (Troncoso et al., 2007). This suggests that whisker movements are subject to change following long-term synaptic plasticity in wM1. Although complete ablation of wM1 does not abolish whisking, it does disrupt whisking kinematics, coordination, and temporal organization such as whisking synchrony (Gao et al., 2003). There are several indirect routes from wM1 to the lateral facial nucleus, for example via SC (see Superior Colliculus) or the pontomedullary RF (see Pontomedullary Reticular Formation) and wS1 (see Somatosensory Cortex as a Premotor Area). In addition, wM1 is involved in several feedback loops, including reciprocal connections with wS1 (Aronoff et al., 2010), thalamus (Cicirata et al., 1986; Colechio and Alloway, 2009), and loops involving the basal ganglia (see Basal Ganglia), the cerebellum (see The Cerebellar System), and the claustrum (see Bilateral Coordination of Whisker Movements). Finally, wM1 projects to the deep mesencephalic nucleus, the periaqueductal gray, and the red nucleus (Alloway et al., 2010). This network of inputs and outputs enables wM1 to adjust whisker movements both to sensory input and to the general behavior.

The output of wM1 is not uniform. Layer 5 pyramidal cells project to cells around the facial nucleus while those of layer 6 project to the thalamus. Evidence for strong myelinization and an expanded layer 5 in AGm points to the possible contribution to high speed whisking (Brecht et al., 2004b). Layer 5 output may correspond with timing of individual whisking movements and may be able to reset these rhythms, while layer 6 output may correspond with grouping of multiple whisking movement bursts where action potential frequency determines movement direction and amplitude (Brecht et al., 2004b).

**Somatosensory cortex as a premotor area**

Microstimulation of wM1 can induce both whisker protraction and retraction depending on the location of stimulation in wM1 (Gioanni and Lamarche, 1985; Haiss and Schwarz, 2005; Matyas et al., 2010). A recent study found that stimulation of wS1 induces whisker retraction at shorter latencies than wM1 stimulation. In fact, the wM1-induced whisker retraction can be mediated by synaptic activation in wS1 (Matyas et al., 2010). Contrary to stimulation of wM1, stimulation of wS1 does not evoke whisker protraction (Matyas et al., 2010). In the same study, the authors suggest that wS1 exerts its effect on whisker movement by a disynaptic pathway via SpV to the facial nucleus. Thus, wM1 and wS1 could together form an additional source of rhythmic whisker movements, alongside the putative brainstem pattern generators (see Serotonin). Such an organization is in line with the idea that wM1 specifies motor programs rather than simple muscle activity (Brecht et al., 2004b).

**BASAL GANGLIA**

The first somatosensory feedback system to be discussed involves the basal ganglia, which are important for a wide variety of (sensori-)motor functions. In the oculomotor system, the basal ganglia have been associated with orienting saccadic eye movements based on reward expectancy (Hikosaka et al., 2006). A similar function for the whisker system could very well be possible. The basal ganglia are a heterogeneous group of brain regions, whose main components are the striatum, the globus pallidus (GP), the substantia nigra (SN), and the subthalamic nucleus (STN). The GP consists of two parts: an external (GPe) and an internal part (GPI). In rodents, GPI is commonly referred to as the entopeduncular nucleus (EPN; Nambu, 2007). SN is composed of a pars compacta (SNc) and a pars reticulata (SNr). In general, the information from the cerebral cortex enters the striatum, is forwarded to other parts of the basal ganglia and the output to the thalamus and SC is eventually generated by EPN and SNr (Figure 5A).

The striatum, or “neostriatum,” is a single area in rodents, but in higher mammals it is composed of two nuclei: the caudate and the putamen (Tepper et al., 2007). Based on function and connectivity, the striatum can be divided into a dorsolateral and a ventromedial part (Voorn et al., 2004). The striatum is involved in the acquisition of habits, goal-directed behaviors and in the motivation to perform. The whisker receptive fields of the dorsolateral striatum are organized in a loosely somatotopic manner: dorsal whiskers project laterally and caudal whiskers project dor-sally (Figure 5B; Alloway et al., 1999; Wright et al., 1999). There is much overlap between the projection areas of whiskers from a single row, but hardly any from whiskers in different rows. There is also a weaker whisker representation in the ventromedial striatum (Alloway et al., 1999; Wright et al., 1999). The cortico-striatal projections are predominantly ipsilateral and originate from layer 5 pyramidal cells in both barrels and septa (Alloway et al., 2006). Thus, cortico-striatal projections serve to integrate rather than segregate input from different whiskers. In addition, the striatum receives input from wS2, wM1, and other cortical areas, including motor, cognitive, and other sensory areas (Wright et al., 2001; Alloway et al., 2006; Tepper et al., 2007). Hence, the striatum can integrate the whiskers and general behavior.

Apart from the extensive input from the cerebral cortex, the striatum also receives direct input from the thalamus. The thalamo-striatal connections mainly lie in the intralaminar nuclei of the thalamus (Smith et al., 2004; Tepper et al., 2007) and in Pom (Alloway et al., 2006). During whisker stimulation at low frequencies, the responses of the medium-spiny neurons in the dorsolateral striatum are approximately 5 ms later than in wS1 (Mowery et al., 2011; Pidoux et al., 2011; Syed et al., 2011). However, during repeated whisker stimulation at 5–8 Hz, striatal responses actually preceded those in wS1 (Mowery et al., 2011). In addition, the striatal responses showed less adaptation to repeated
whisker stimulation as responses in wS1 (Mowery et al., 2011). The latter findings support an important role for the direct thalamo-striatal pathway, in addition to the well-established thalamo-cortical-striatal route. The thalamo-striatal pathway conveying whisker information originates mainly in Pom (Allloway et al., 2006). Relay cells in Pom are inhibited during rest and become disinhibited during periods of activity (see Zona Incerta and Anterior Pretectal Nucleus). Although the disinhibition of Pom has been predominantly linked to active whisking (Bokor et al., 2005; Lavallée et al., 2005; Urbain and Deschênes, 2007a), it might also be evoked by repeated, passive whisker input. Other inputs to the striatum come from the amygdala (Kelley et al., 1982; Popescu et al., 2009), the dorsal raphe nuclei (Di Matteo et al., 2008), GP and SN (Tepper et al., 2007). The main output of the striatum is composed of GABAergic projections to GP and SN.

The GABAergic output of the striatum is the dominant input to SNc, but SNc also receives GABAergic input from SNr and glutamatergic input from the amygdala, and to a lesser extent also from STN (Kita and Kitai, 1987; Gonzales and Chesselet, 1990; Misgeld, 2004). SNc also receives GABAergic input from the tuberomammillary nuclei (Lee et al., 2008b). SNc forms dopaminergic connections to the striatum and is implicated in the reward system (Hikosaka et al., 2006; Redgrave et al., 2008). Its degeneration is an important cause of the motor problems associated with Parkinson’s disease (Gibb and Lees, 1980; Esposito et al., 2007). SNr receives GABAergic input from the striatum and, to a lesser extent also glutamatergic input from STN and the cerebral cortex (Kita and Kitai, 1987; Naito and Kita, 1994; Tepper et al., 2007). SNr sends GABAergic projections to the ventromedial thalamus and the SC (Beckstead et al., 1979; Di Chiara et al., 1979; Grofova et al., 1982). Activation of the nociceptin/orphanin FQ (N/OFQ) receptors in SNr modulates whisker motor output (Marti et al., 2009).

The external globus pallidus receives GABAergic input from the striatum and glutamatergic input from STN. Sparse innervation comes from the cerebral cortex, the intralaminar nuclei of the thalamus, SNc, the dorsal raphe nuclei, and PPTg (Kita, 2007). The main output of the basal ganglia is directed to the thalamus, via GPe and SNr, and the superior colliculus, via SNr. The line thickness indicates the relative importance for the whisker system. (B) (Whisker responses in the dorsolateral (dl) striatum follow a loose somatotopy, which is mainly organized according to whisker rows. The black dots indicate schematically the projections of the layer 5 pyramidal cells in the B2 barrel of left wS1. The projection is main, but not exclusively, ipsilateral, and largely within the “B row” area in the striatum. There is considerable overlap, however, with the projection areas of other B row whiskers. “Rostral” and “caudal” refer to the positions of the whiskers on the mystacial pad. A smaller and less characterized projection area is also present in the ventromedial (vm) striatum.

superior colliculus

The second sensorimotor feedback system involves SC, which is also known as the “tectum.” The upper layers of SC process sensory information, the intermediate layers sensorimotor information, and the lower layers motor information. SC receives sensory input...
via direct connections from all four parts of the sensory trigeminal nuclei (Steindler, 1985; Cohen et al., 2008), and provides a direct output to the facial nucleus. However, the SC neurons that receive trigeminal input are not the same as those that innervate the facial nucleus (Hemelt and Keller, 2008). Hence, SC does not function as a simple, "reflexive" relay station between the trigeminal nuclei and the facial nucleus. Similarly, the input to SC from wM1 is also not directly relayed to the facial nucleus, since microstimulation of wM1 and SC show qualitatively and quantitatively different whisker responses (Hemelt and Keller, 2008). Instead, the main function of SC for the whisker system may be closely related to its best known function, which is to control saccadic eye movements and direct the gaze direction toward an interesting visual cue (Boehnke and Munoz, 2008; Gandhi and Katnani, 2011). SC can direct all mobile senses toward an object of interest. Microstimulation at a single spot in the intermediate or deep layers of SC can induce coherent movements of the eyes, the auricles, and the whiskers together (McHaffie and Stein, 1982).

While microstimulation within wM1 induces rhythmic whisker movements (Brecht et al., 2004b; Matyas et al., 2010), microstimulation of the whiskers and the SC together (McHaffie and Stein, 1982) or within the SC alone (Hemelt and Keller, 2008), which is in accordance with its putative function in the direction of the whiskers. In addition, SC also responds to whisker input. Passive touch (air puff) as well as whisking in air and active touch (surface contact during whisking) evoked SC neuronal responses which were subject to strong adaptation. Passive and active touch evoked stronger responses than whisking in air. As a consequence, whisking in air at 10 Hz hardly evokes any response in SC, but active touch does (Bezdudnaya and Castro-Alamancos, 2011). SC responses can have different latencies. Fast responses (<10 ms) are probably due to the direct trigemino-tectal input and slow responses are likely mediated by wS1 (Bezdudnaya and Castro-Alamancos, 2011).

The superior colliculus receives strong input from ipsilateral wM1 (Miyashita et al., 1994; Alloway et al., 2010), wS1 (Wise and Jones, 1977; Cohen et al., 2008; Aronoff et al., 2010) and the cerebellar nuclei, mainly the dentate and interpositus nuclei, and to a lesser extent also from the fastigial nucleus (May, 2006). Other inputs come, as mentioned before, from the trigeminal nuclei (Steindler, 1985; Cohen et al., 2008), and also from ZI, which supplies both glutamatergic and GABAergic efferents (Beitz, 1989; Kim et al., 1992), from SNr (Beckstead et al., 1979; Kaneda et al., 2008) as well as from the visual cortex (Boehnke and Munoz, 2008). There is also input from the thalamus, but this seems to relate more to the visual than to the whisker system (Gosenza and Moore, 1984; Taylor and Lieberman, 1987). SC projects to the lateral facial nucleus. This connection is mainly ipsilateral, but there are distinct patches of neurons within SC that project to the contralateral lateral facial nucleus (Hemelt and Keller, 2008). SC also projects to the contralateral nucleus reticularis tegmenti pontis (NRT; Westby et al., 1993; May, 2006), which provides mossy fiber input to the cerebellar cortex and cerebellar nuclei (Mihaloff, 1993). There is also a projection from SC to the contralateral medial accessory olive (MAO; Huerta et al., 1983; May, 2006), which is a source of climbing fibers to the cerebellar cortex. Thus, there are two disinaptic pathways from SC to the cerebellar cortex, which projects back to SC via the cerebellar nuclei.

THE CEREBELLAR SYSTEM

The third somatosensory feedback system is that of the cerebellum, which receives most of its mossy fiber afferents from the pons and all its climbing fiber afferents from IO (Figure 6).

The pontine nucleus and the nucleus reticularis tegmenti pontis

The pontine nucleus (or “basal pontis”) forms the main gateway to the cerebellum for efferents from the cerebral cortex. The main input to the pontine nucleus comes from layer 5 neurons
The cerebellum has a central role in sensorimotor integration and all go to the cerebellum (Legg et al., 1989; Brodal and Bjaalie, 1992). Cerebral cortical inputs are mapped multiply and in different combinations to the pontine nucleus (Schwarz and Möck, 2001; Leergaard et al., 2004, 2006). In general, cortico-pontine projections from different cortical regions do not overlap. This seems to hold true also for the barrels of wS1, implying that the pontine nucleus may receive single-whisker input (Schwarz and Möck, 2001). Nevertheless, the whisker-related parts of wS1, wS2, and wM1, sometimes project to adjacent, or even partially overlapping regions (Leergaard et al., 2004). Thus, the somatotopy in the pontine nucleus is somewhat intermediate between the continuous somatotopy of the cerebral cortex and the fractured somatotopy of the cerebellum.

The pontine nucleus sends bilateral (but mainly contralateral) mossy fiber connections to the cerebellar cortex, which give off collaterals to the cerebellar nuclei (Eiller and Chan-Palay, 1976; Parenti et al., 2002; Leergaard et al., 2006). The cerebellar cortex also projects to the cerebellar nuclei. This feedback loop is completed by afferents from the cerebellar nuclei back to the pontine nucleus (De Zeeuw et al., 2011; Ruigrok, 2011). In addition to the input from the cerebral cortex, which is the dominant input, and of the cerebellum, the pontine nucleus also receives inputs from dozens of other brain regions (Mihailoff et al., 1989). The functional relevance of these other inputs is not very clear, and their specific functions for the whisker system are currently unknown.

The inputs that could be of importance for the whisker system include projections from the sensory trigeminal nucleus (mainly SpVi; Swenson et al., 1984; Mihailoff et al., 1989), SC (Burne et al., 1981; Mihailoff et al., 1989), ZI (Ricardo, 1981; Mihailoff, 1993), the dorsal raphe nuclei (Mihailoff et al., 1989), PPTg (Mihailoff et al., 1989), and the tuberomammillary nuclei (Pilott et al., 2002). Recently, a direct connection from STN to the pontine nuclei has been described in cebus monkeys (Rostan et al., 2010). This could underlie a direct coupling between the basal ganglia and the cerebellar system.

Immediately dorsal of the pontine nucleus is the NRTP. The main input to NRTP comes from the cerebellar nuclei (Torigoe et al., 1986b; Brodal and Bjaalie, 1992). Other inputs come from SC and the pontomedullary RF (Torigoe et al., 1986b). NRTP also receives input from layer 5 pyramidal cells of the cerebral cortex, mainly bilaterally from the cingulate cortex and to a lesser extent also ipsilaterally from motor areas (Brodal, 1980; Torigoe et al., 1986a). NRTP projects, amongst others, ipsilaterally to the cerebellar cortex and the cerebellar nuclei (Mihailoff, 1993; Parenti et al., 2002) and bilaterally to the lateral facial nucleus (Isokawa-Akesson and Komisaruk, 1987; Hattox et al., 2002). Hence, NRTP may be a relay station between the cerebellar nuclei and the lateral facial nucleus, but whether it has a role in the whisker system is not clear yet.

Cerebellum and inferior olive

The cerebellum has a central role in sensorimotor integration and motor learning (Ito, 2000; De Zeeuw and Yeo, 2005; Krakauer and Shadmehr, 2006). It receives sensory input from the whiskers (Figure 6B; Axelrad and Crepel, 1977; Brown and Bower, 2001; Loewenstein et al., 2005; Bosman et al., 2010; Chu et al., 2011) and its activity can affect whisker movements (Esakov and Pronichev, 2001; Lang et al., 2006). The cerebellar cortex has two afferent pathways, the climbing fiber and mossy fiber/parallel fiber pathway, that converge on the cerebellar Purkinje cells, which form the sole efferent projection to the cerebellar and vestibular nuclei (De Zeeuw et al., 2011).

Each adult Purkinje cell is innervated by a single climbing fiber only, with the climbing fiber-to-Purkinje cell synapse being extraordinarily strong (Eccles et al., 1964; Bosman et al., 2008; Davie et al., 2008). Thus, climbing fiber activity reliably evokes postsynaptic spikes, which are, due to their complex waveforms, called “complex spikes” (Davie et al., 2008; De Zeeuw et al., 2011). Climbing fibers originate exclusively from the contralateral IO. IO comprises three main nuclei, all of which receive input from SpV, but not from PrV (Molinari et al., 1996; Yatim et al., 1996). Trigemino-olivary connections originate from all three compartments of SpV and target mainly the contralateral rostromedial part of the dorsal accessory olive (DAO) and the adjacent dorsal leaf of the principal olive (PO), and to a lesser extent the ventral leaf of the PO and the caudal part of the MAO (Huerta et al., 1983; Molinari et al., 1996; Yatim et al., 1996). Ipsilateral trigemino-olivary projections mirror the contralateral ones, but are relatively sparse (Molinari et al., 1996; Yatim et al., 1996). Altogether, most IO neurons react to somatosensory input (Gellman et al., 1985; Gibson et al., 2004). IO also receives input from many other regions. These include direct and indirect spinal projections (Miskolczy, 1931; Swenson and Castro, 1983), as well as projections from SC (Akaike, 1992), Z1 (Brown et al., 1977), the raphe nuclei (Brown et al., 1977), and the ipsilateral cerebral cortex, both from somatosensory and motor areas (Swenson et al., 1989). As a consequence, Purkinje cells fire complex spikes in response to stimulation of wM1 (Lang, 2002; Lang et al., 2006).

The subnuclei of IO project to specific parasagittal zones of the cerebellar cortex (Voogd and Glickstein, 1996; Apps and Hawkes, 2009). The IO area with the strongest trigeminal input, the rostro-medial DAO and dorsal PO, projects to the C3 and D zones, while the other areas project mainly to the A zones (Yatim et al., 1996; Apps and Hawkes, 2009). Indeed, most Purkinje cells showing complex spike responses to whisker stimulation were found in the C3 and D zones in lobule crus 1, and to a lesser extent also in crus 2 (Figure 6C; Bosman et al., 2010). Climbing fiber responses have also been found in the A zones of lobule VII (Thomson et al., 1989). In lobule IX, mossy fiber whisker responses have been reported, but climbing fiber responses were not evaluated (Joseph et al., 1978).

Climbing fiber input to the cerebellar cortex does not follow a somatotopic organization on single-whisker level. For most Purkinje cells, the receptive field of the climbing fiber input is restricted to a single whisker, where nearby Purkinje cells may receive inputs from totally unrelated whiskers (Axelrad and Crepel, 1977; Bosman et al., 2010). In the rare cases where a Purkinje cell received input from multiple whiskers, these whiskers were located within the same row (Bosman et al., 2010). Complex spike responses to whisker stimulation are relatively sparse, encoding typically about 10% of the stimuli in responsive Purkinje cells, show a large jitter in the latencies and depend on the direction of whisker movement (Thomson et al., 1989; Bosman et al., 2010).
Mossy fibers terminate at the cerebellar granule cells, whose axons form the parallel fibers, that run transversely over a long distance, innervating numerous Purkinje cells on their way, but with each parallel fiber-to-Purkinje cell synapse being only very weak (De Zeeuw et al., 2011). There are two main mossy fiber routes via which whisker sensory information reaches the cerebellar cortex. First, there is a direct mossy fiber projection from the trigeminal nuclei to the cerebellar cortex. The trigemino-cerebellar mossy fibers originate from ipsilateral PrV, SpVo, and SpVi, and to a lesser extent from SpVc (Yatim et al., 1996). This direct pathway can evoke Purkinje cell simple spike responses with a short latency. The second main mossy fiber input originates in the pontine nucleus, which in turn is activated by wS1. This cerebro-cerebellar pathway evokes Purkinje cell simple spike responses with a long latency. Lesioning of the cerebral cortex abolishes the long-latency response, while leaving the short-latency responses in tact (Figure 6A; Kennedy et al., 1986; Morisette and Bower, 1996). There is also a direct, trigemino-pontine connection from SpVi, but its relevance for the whisker system is not clear (Swenson et al., 1984; Mihailoff et al., 1989).

Whisker input can also inhibit Purkinje cell simple spike firing, with the inhibitory response having a longer latency than the excitatory response (Figure 6B; Bosman et al., 2010; Chu et al., 2011). This reflects most likely the feedforward inhibition by molecular layer interneurons within the cerebellar cortex (Chu et al., 2011; De Zeeuw et al., 2011). The complex spike and simple spike responses of an individual Purkinje cell are largely uncorrelated, both at the level of the receptive field and on the level of individual trials (Bosman et al., 2010). Simple spikes receptive fields usually involve multiple whiskers, without any obvious somatotopic ordering. And, also in contrast to complex spike responses, simple spike responses are not affected by the direction of whisker stimulation (Bosman et al., 2010). Mossy fiber-mediated whisker input seems to be strongest in crus 1, strong in crus 2 and lobules VII and IX in the vermis, and sparse in the simplex and paramedian lobules (Joseph et al., 1978; Shambes et al., 1978; Thomson et al., 1989; Bosman et al., 2010).

Thus, large parts of the cerebellar cortex receive whisker input. The output of the GABAergic Purkinje cells in the whiskersensitive regions is fully directed to the cerebellar nuclei. From there, the cerebellar output to the whisker system mainly follows three pathways: (i) to IO, where it closes the olivo-cortico-nuclear feedback loop (Voogd and Glickstein, 1998; De Zeeuw et al., 2011); (ii) to the VL nucleus of the thalamus (Aumann et al., 1994) to provide feedback to the cerebral cortex (Aumann et al., 1994), and possibly also to the basal ganglia (Hoshi et al., 2005); and (iii) to regions that directly project to the lateral facial nucleus, such as SC (Weisbly et al., 1993) and NRT (Torigoe et al., 1986b). So these latter routes may allow the cerebellum to directly affect motor output.

Both the striatum and the pontine nuclei receive input from the cerebral cortex. Interestingly, the cortico-pontine pathway has a stronger convergence of inputs from related regions in wS1 and wS2 than the cortico-striatal pathway (Leergaard et al., 2004). This could imply that the cerebellar system is especially suited for the processing of sensory data. Recent findings in primates link the cerebellar system and the basal ganglia via reciprocal disynaptic pathways. The dentate nucleus projects via the thalamus to the striatum (Hoshi et al., 2005) and STN projects via the pontine nuclei to the cerebellar cortex (Bostan et al., 2010).

Ventralateral nucleus of the thalamus
Both the basal ganglia and the cerebellum have an ascending projection to wM1 via the ventrolateral nucleus (VL) of the thalamus. VL incorporates input from EPN (Nambu, 2007), the cerebellar nuclei (Aumann et al., 1994), and wM1 (Miyashita et al., 1994; Alloway et al., 2008). VL itself has a somatotopic representation, including a separate area related to the whiskers (Tamas and Brumberg, 2010). Thus, VL is a crucial part of the central motor control system.

Other structures projecting to the facial nucleus
Pontomedullary reticular formation
The pontomedullary RF is a premotor area, whose activation can cause widespread movements (Quesy and Freedman, 2004; Stapley and Drew, 2009). Within RF, several distinct regions can be discriminated. Of these, the dorsal medullary reticular field and the parvocellular reticular nucleus receive strong input from SpVi and SpVc, while the gigantocellular reticular nucleus receives moderate input from SpVo (Zerari-Mailly et al., 2001). Relatively weak inputs from SpV to the other parts of RF can also be found, as well as a few connections between PrV and RF (Zerari-Mailly et al., 2001). The dorsal reticular nucleus (DRN) is probably a pain modulating area (Villanueva et al., 1988; Boudassra et al., 1992). DRN forms, as other parts of RF, strong bilateral connections to the facial nucleus (Hattox et al., 2002; Leite-Almeida et al., 2006). Indeed, mice move their whiskers, as well as other parts of the face, in response to pain (Langford et al., 2010). In addition, DRN projects to dozens of other brain structures, including other parts of RF, the ipsilateral amygdala, periaqueductal gray, red nucleus, and SpV, as well as the contralateral IO, SC, ZI, and several nuclei of the thalamus, including Pom and to a lesser extent VPM (Leite-Almeida et al., 2006). Apart from a role in pain transmission, RF is also involved in “normal” whisker movements. RF neurons receiving trigeminal input project to the lateral facial and hypoglossal nuclei (Dauvergne et al., 2001). In addition, RF receives direct input from wM1, and RF stimulation causes whisker retraction (Matyas et al., 2010). RF also receives cholinergic input from the pedunculopontine tegmental nuclei (Jones, 1990) and noradrenergic input from the locus coeruleus (Jones, 1991), indicating that RF activity is strongly modulated by the general state of alertness.

Zona incerta
The zona incerta can be functionally divided into rostral (ZIr), dorsal (ZId), ventral (ZIv), and caudal (ZIc) sectors (Kim et al., 1992; Ma et al., 1992; Nicoléls et al., 1992, 1995b) and contributes to the whisker paralemniscal somatosensory pathway (Urbain and Deschênes, 2007a). It has been said to have connections with almost every center in the neuraxis (Mitrofanis, 2005). Multiple whisker receptive fields have been found in both ZId and ZIv. A somatotopic map was found to be partial in ZId and complete in ZIv. The ZId somatotopic map was characterized by large facial receptive fields including the whiskers (Nicoléls et al., 1992; Simpson et al., 2008). Direct whisker input reaches ZI mainly from both
Pom, providing a mechanism of lateral inhibition in ZI (Urbain et al., 2004). Thus, during active whisker movements, wM1 activity releases the inhibition on sensory gating in Pom. This implies that Pom transmits more details on whisker input during active movement than during periods of rest. Apart from wM1, also cholinergic input from PPTg and the laterodorsal tegmental nucleus (LDTg) can reduce the inhibitory output of Zlv to Pom (Trageser et al., 2006). Since the cholinergic input to ZI is highest during active states (Trageser et al., 2006), this is a possible second form of gating of the whisker input to wS1 under control of ZI. ZI forms also GABAergic projections to the intermediate and deep layers of SC, that in turn project back to ZI (Roger and Cadusseau, 1985; May, 2006). In addition, Zld has glutamatergic projections to the basal ganglia (Heine and Mitrofanis, 2004).

Anterior pretectal nucleus
Like ZI, the APT nucleus provides strong GABAergic inhibition in Pom. The morphology of the projections to Pom from ZI and APT are similar, forming multiple synapses on the thick dendrites of relay cells, and different from RT projections that form single synapses on the thin, distal dendrites of relay cells (Bokor et al., 2005; Wanaverbecq et al., 2008). Input from APT strongly suppresses whisker responses in Pom (Murray et al., 2010). In view of the heterogeneity in firing patterns of APT neurons observed in vivo, it has been suggested that APT, like ZI, controls the thalamo-cortical output in a state-dependent manner (Bokor et al., 2005). ZI and APT are reciprocally connected. There is a strong projection of both GABAergic and non-GABAergic APT neurons to Zlv, from which the thalamic projections originate (May et al., 1997). The reciprocal connection from Zlv to APT is relatively sparse (May et al., 1997; Giber et al., 2008). Thus, ZI and APT may cooperate in controlling the flow of information from Pom to the cerebral cortex in a state-dependent manner.

Apart from ZI and Pom, APT also targets a large number of brain regions. The functional relevance of these other outputs for the whisker system is still unclear, but potentially relevant target areas are SC, the pontomesencephalic RF, the pontine nucleus, red nucleus, and (dorsal) IO (Cadusseau and Roger, 1991; Terenzi et al., 1995; Zagon et al., 1995). In turn, APT receives strong input from amongst others wS1, SC, the deep mesencephalic nucleus, and PPTg, as well as sparse input from the locus coeruleus and the periaqueductal gray (Foster et al., 1989; Cadusseau and Roger, 1991).

Red nucleus
The red nucleus is closely associated with limb movements (Massion, 1988; Muir and Whishaw, 2000) and is composed of two parts. The magnocellular part receives input from the cerebral cortex, including wS1 and wM1 (Alloway et al., 2010), as well as from the cerebellar interposed nucleus (Teune et al., 2000), and sends its output to the contralateral limbs via the rubrospinal tract (ten Donkelaar, 1988; Paul and Gould, 2010). The parvocellular part receives its input from the cerebellar dentate nucleus (Teune et al., 2000) and sends its output to the contralateral facial nucleus (Hattox et al., 2002). Thus, from an anatomical point of view, the red nucleus is strongly implicated in the whisker system. However, electrical stimulation of the red nucleus did not evoke whisker movements in a consistent way (Isokawa-Akesson and Komisaruk, 1987).

Pontine respiratory group
The parabrachial complex and the Kölliker-Fuse nucleus, both part of the pontine respiratory group, provide strong, ipsilateral projections to the lateral facial nucleus (Isokawa-Akesson and Komisaruk, 1987; Hattox et al., 2002). The pontine respiratory group projects to several areas of the medullar respiratory group, and may therefore affect the respiratory rhythm (Smith et al., 2009). Thus, the connection between the pontine respiratory group and the lateral facial nucleus may facilitate the synchronization of sniffing and whisking. Such coupling is prominent during exploratory whisking (Welker, 1964).

Ambiguous nucleus
The ambiguous nucleus has a dense projection to the ipsilateral lateral facial nucleus (Isokawa-Akesson and Komisaruk, 1987; Hattox et al., 2002). Electrical stimulation of the ambiguous nucleus could evoke ipsilateral, rhythmic whisker movements with a remarkably low stimulation threshold (Isokawa-Akesson and Komisaruk, 1987). Since the ambiguous nucleus is mainly involved in respiration (Delgado-García et al., 1983) and swallowing (Broussard and Altschuler, 2000), it could serve to synchronize whisker movements to respiration and swallowing.

Other brain regions
This list of brain regions is incomplete since we lack sufficient knowledge of other brain regions which might be involved in the whisker system. Potentially important areas include the deep mesencephalic nucleus and the periaqueductal gray. The deep mesencephalic nucleus receives a strong, ipsilateral input from wM1, and forms dense projections to the lateral facial nucleus (Hattox et al., 2002; Alloway et al., 2010). Yet, its function for the whisker system is not clear. The periaqueductal gray is, amongst others, important for pain transmission and integrating defensive behavior (Behbehani, 1995; Graeff, 2004). Stimulating the periaqueductal gray results in whisker twitches (Verberne and Struyker Boudier, 1991). The periaqueductal gray receives serotonergic input from the dorsal raphe nucleus (Graeff, 2004), strong input from ipsilateral wM1 (Alloway et al., 2010), and forms relatively sparse, bilateral connections to the lateral facial nucleus (Hattox et al., 2002). For further connections of the periaqueductal gray, see Vianna and Brandão (2003).
Chapter 1

**BILATERAL COORDINATION OF WHISKER MOVEMENTS**

In the absence of object contact and head movements, whisker movements on both sides of the head tend to be symmetric. However, during active exploration, in particular involving head movements, whisker movements are often asymmetric (Towal and Hartmann, 2006, 2008; Mitchinson et al., 2007). This implies that both hemispheres are interconnected, but can be decoupled if the actual behavior requires to do so. In line with this, many, if not most, of the connections discussed in this review are actually bilateral, although the strengths of the ipsi- and contralateral projections are often quite different (see also Alloway et al., 2010). Putative candidates for the modification of interhemispheric connections, especially involving wM1, are the feedback loops with the thalamus, the basal ganglia, and the claustrum (Alloway et al., 2008, 2009, 2010). Particularly the claustrum has been proposed to facilitate interhemispheric communication of wM1 (Alloway et al., 2009; Smith and Alloway, 2010). wM1 targets the claustrum mainly contralaterally, and the claustrum projects mainly ipsilaterally to wM1. These projections are highly specific: the cortico-claustral-cortical projections connect the same whisker fields in wM1 of both hemispheres (Smith and Alloway, 2010).

**AROUSAL, ALERTNESS, AND ATTENTION**

Whisker movements and the processing of whisker input depend on the general state of alertness. For instance, stimulation of wM1 leads to larger whisker movements in aroused rather than in awake, but sessile rats (Berg et al., 2005). Furthermore, whisker stimuli evoke smaller responses in wS1 showing less spreading during whisking than during rest (Ferezou et al., 2007). The neural systems that control the general state of alertness affect many brain regions and are not specific for the whisker system. We discuss here those systems of which clear effects on the whisker system have been documented or can be expected based on anatomical connections.

**ACETYLCHOLINE**

Central cholinergic projections, mainly originating from the basal forebrain and the tegmentum, affect the whisker system at different levels (Woollf, 1991; Dani and Bertrand, 2007). Roughly, the basal forebrain targets wS1 and wM1, while the tegmentum targets several subcortical areas. The basal forebrain is composed of several areas that provide cholinergic output, including the nucleus basalis magnocellularis (NBM; known as the Meynert nucleus in primates) of the substantia innominata. NBM is active during waking and REM sleep, but not during slow-wave sleep (Lee et al., 2005). The main projection areas of the cholinergic neurons of NBM are the entire cerebral cortex and the amygdala (Wenk, 1997; Deurselver and Semba, 2011). Electrical stimulation of the cholinergic neurons of the NBM leads to an increased effect of wM1 stimulation on whisker movements. This effect of NBM stimulation is only observed in sessile, but less so in aroused rats (Iberg et al., 2005). This could indicate that NBM is already endogenously active in aroused rats.

In addition to enhancing motor performance, cholinergic afferents also increase the sensitivity to sensory stimuli. The response to whisker stimulation in wS1 is increased due to acetylcholine (ACh; Oldford and Castro-Alamancos, 2003; Constantinople and Bruno, 2011). This effect is partly due to stimulation of the basal forebrain, which enhances especially the responses to non-dominant whiskers (Kuo et al., 2009). In addition, cholinergic projections from PPTg and LDTg, increase the responses to whisker stimulation in VPM, and consequently also in wS1 (Hirata and Castro-Alamancos, 2010, 2011). Furthermore, the responsiveness to whisker input of Pom is increased by cholinergic input from PPTg as well as from LDTg, due to both direct connections to Pom, where the cholinergic fibers suppress the release of GABA from projections originating in ZI (Marsi et al., 2006), as well as indirectly by decreasing the neuronal activity of GABAergic projections onto Pom (Hirata et al., 2006). In SpVi, a similar phenomenon occurs as in wS1: activity of the cholinergic input from PPTg increases the responsiveness of sensory neurons to inputs from adjacent whiskers (Timofeeva et al., 2005). Finally, there are also cholinergic projections from PPTg and LDTg to SC, PrV, and the lateral facial nucleus (Satoh and Fibiger, 1986; Beak et al., 2010), but their specific functions for the whisker system are currently unknown.

The nucleus basalis magnocellularis receives strong input from the amygdala, the hypothalamus, and the thalamus, as well as from specific areas of the cerebral cortex, probably including the prefrontal and motor cortex (Haring and Wang, 1986; Irle and Markowitsch, 1986). In addition, there are weaker inputs from many other (subcortical) regions (Haring and Wang, 1986; Irle and Markowitsch, 1986). Inputs to PPTg and LDTg come from a wide range of brain regions, including the medial prefrontal and cingulate cortex (but not wS1, wS2, and wM1), the thalamus, the hypothalamus, ZI, the periaqueductal gray, SC, the pontomedullary RF, the dorsal raphe nuclei as well as from many other regions not directly involved in the whisker system (Semba and Fibiger, 1992). The input from the trigeminal and cerebellar nuclei is relatively weak (Semba and Fibiger, 1992).

In conclusion, the cholinergic system facilitates whisker movements during arousal, which increases the sensitivity of the whisker system even further.

**NORADRENALINE**

Noradrenergic projections have similar effects on the sensitivity to whisker stimulation as cholinergic projections. The origin of noradrenaline is the locus coeruleus and adjacent brainstem regions (Aston-Jones and Cohen, 2005). Noradrenaline suppresses spontaneous activity of VPM via RT. As a consequence, sensory input is passed on to wS1 with a higher signal-to-noise ratio (Hirata et al., 2006; Hirata and Castro-Alamancos, 2011). In addition, the locus coeruleus can directly modulate the network dynamics of wS1 (Constantinople and Bruno, 2011). Activity of the locus coeruleus is closely related to awareness and alertness (Aston-Jones and Cohen, 2005). Indeed, a novel environment can stimulate activity of the locus coeruleus and the anterior cingulate cortex, and thus keep the animal fully awake (Gompf et al., 2010). The main inputs to the locus coeruleus come from RF and the hypoglossal nucleus.
(Jones, 1991). Other relevant outputs are directed to RF, the facial nucleus, ZI, and NBM (Jones, 1991). Thus, although noradrenaline works via different mechanisms than ACh, both increase the level of arousal as well as the sensitivity toward whisker input.

HISTAMINE
Histamine is also only released during wakefulness (Takahashi et al., 2006). It promotes, amongst others, vigilance (Anaclet et al., 2009; Thakkar, 2011) and the coordination of goal-directed behaviors (Valdes et al., 2010). The sole source of histamine in the brain is the hypothalamus, most notably the tuberomammillary nuclei and perhaps also the surrounding tissue (Wouterlood et al., 1986; Passani and Blandina, 2011). The tuberomammillary nuclei project to almost all brain regions, including cerebral cortex, thalamus, brainstem, and cerebellum (Pillot et al., 2002). Histaminergic connections of particular importance for the whisker system include ipsilateral projections from the ventrolateral tuberomammillary nucleus to wS1 and wM1 (Hong et al., 2010). The dorsomedial tuberomammillary nucleus projects bilaterally to PrV and the lateral facial nucleus (Hong et al., 2010). In addition, all layers of SC, but mainly the superficial ones, receive histaminergic input (Manning et al., 1996). Thus, there are histaminergic connections to many of the important whisker regions, and although the specific functions of these connections are currently unknown, it seems likely that histamine has a general, stimulating effect on the whisker system, comparable to that of acetylcholine and noradrenaline.

SEROTONIN
The activity of most serotonergic neurons of the dorsal raphe nucleus is strongly affected by the sleep/wake rhythm. In the awake state, they fire at very regular intervals (McCinty and Harper, 1976; Kosciu et al., 2006; Urbain et al., 2006). The dorsal raphe nucleus projects to the lateral facial nucleus (Hattox et al., 2003; Cramer and Keller, 2006; Lee et al., 2008c). Serotonin facilitates a persistent inward current (PIC) in the whisker motor neurons. This lowers their activation thresholds (Cramer et al., 2007). Indeed, spontaneous as well as wM1-induced whisker movements are largely abolished following block of serotonin receptors (Figure 7B; Hattox et al., 2003; Cramer and Keller, 2006). Thus, serotonin is both required and sufficient to generate a rhythmic whisker movement pattern, and it also modulates inputs from wM1. That makes the serotonergic system a fourth system to the brain regions of the whisker system. DR DM, dorsomedial dorsal raphe nucleus; DR VM, ventromedial dorsal raphe nucleus; FN, facial nucleus; PrV, principal trigeminal nucleus; VPM, medial ventroposterior nucleus; wM1, whisker motor cortex; wS1, barrel cortex. (B) During exploratory whisking, rhythmic whisker movements occur, as shown here by EMG recordings of rat whisker muscles. Application of metergoline, an antagonist for the serotonin receptors 5-HT1 and 5-HT2, in the facial nucleus abolishes the rhythmicity of whisker movements unilaterally at the side of injection. Most likely, the dorsal raphe nuclei are the source of serotonin. This indicates that the dorsal raphe nuclei may act as central pattern generator for whisker movements. Reproduced with permission from Hattox et al. (2003).

TIMING IN THE WHISKER SYSTEM
Timing is essential for the whisker system. During active touch, rats move their whiskers rhythmically over an object. Irregularities in the surface texture cause small disruptions in the whisker movements, which evoke neuronal responses (Figures 1B,C; Sowed et al., 2003; Hartmann, 2009; Jadhav and Feldman, 2010). Active touch can be instrumental for several forms of behavior. For instance, Etruscan shrews use their whiskers to locate prey. On
FIGURE 8 | Neuronal connections in the whisker system. Many brain regions are involved in controlling the whiskers. Schematic representation of the connections discussed in the main text. Thickness of the arrows corresponds to the robustness of the connection involved (divided among three different levels). Some local connections are indicated, but for the connections between the nuclei of the basal ganglia, see Figure 5A. Amb, ambiguous nucleus; Amg, amygdala; APT, anterior pretectal nucleus; Clau, claustrum; DMN, deep mesencephalic nucleus; DR, dorsal raphe nucleus; EPN, entopeduncular nucleus; GP, globus pallidus; IO, inferior olive; KF-PC, Kölliker-Fuse nucleus and parabrachial complex; LC, locus coeruleus; LD, laterodorsal nucleus; MeV, mesencephalic trigeminal nucleus; NBM, nucleus basalis magnocellularis; NRTP, nucleus reticularis tegmenti pontis; NXII, hypoglossal nucleus; PAG, periaqueductal gray; PN, pontine nucleus; Pom, medial posterior nucleus; PPTg, pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus; PV, principal trigeminal nucleus; RF, pontomedullar reticular formation; RN, red nucleus; RT, reticular nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SpIV, spinal trigeminal nucleus pars caudalis; SpV, spinal trigeminal nucleus pars interpolaris; SpVI, spinal trigeminal nucleus pars oralis; STN, subthalamic nucleus; TG, trigeminal ganglion; TMN, tuberomammillary nucleus; VPM, medial ventroposterior nucleus; wM1, whisker motor cortex; wS1, barrel cortex; wS2, whisker part of the secondary somatosensory cortex; ZI, zona incerta.
average, they initiate an attack on average 179 ms after the first whisker contact, but this interval can be as short as 53 ms (Munz et al., 2010). Indeed, the vibrissae can be used experimentally to explore interval timing. Stimulation of the vibrissae can act as a CS in eyelid conditioning (Das et al., 2001; Leal–Campanario et al., 2006; Galvez et al., 2009). The reverse is also possible: to evoke vibrissal movements as an unconditioned response (Ur; Trompos et al., 2004).

Whisker responses are rapidly distributed over the brain. Many brain regions receive direct input from the trigeminal nuclei, often in addition to input from wS1 (Figure 8). As a consequence, the whisker responses in the cerebellum (Bosman et al., 2010) and SC (Bezdudnaya and Castro-Alamancos, 2011) are bi-phasic. Fast, direct whisker responses are followed by wS1-mediated responses with longer latencies. This allows for fast, multi-center processing of whisker data.

Although wM1 is able to evoke, on a cycle-by-cycle base, rhythmic whisker movements under experimental conditions involving artificial disinhibition (Castro-Alamancos, 2006), under more physiological conditions, the frequency of microstimulation in wM1 does not necessarily correspond to the frequency of the evoked whisker movements (Berg and Kleinfeld, 2003b; Hass and Schwarz, 2005). However, widespread rhythmic activity (at 7–12 Hz) involving cerebral cortex, thalamus, and brainstem often precedes the onset of rhythmic whisker movements, which is then phase-locked to the brain oscillations (Nicollels et al., 1995a). Nevertheless, it is likely that subcortical structures critically participate in the generation of rhythmicity of the whisker movements. The serotonergic projection from the dorsal raphe nuclei to the facial nucleus has especially been found to be effective in generating rhythmic whisker movements (Hattox et al., 2003). However also the cerebellum and IO may be involved. After blocking IO pharmacologically, as well as following cerebellectomy, the frequency-dependence of whisker movements following wM1 stimulation was altered (Lang et al., 2006). In contrast, SC does not seem to be involved in the generation of rhythmic movements, as its activity causes prolonged whisker protraction (Hemelt and Keller, 2008).

CONCLUSION
Whiskers play a central role in the lives and loves of rodents. Accordingly, many brain regions can affect whisker movements. Whisker movements depend on the general state of arousal, they are coupled to the movements of other mobile senses, like the eyes and the auralis, and integrated with other forms of behavior, like sniffing, swallowing, and locomotion. Animals orient their whiskers based on reward expectancy, for instance when searching for food. With respect to whisker input, the level of detail that is transmitted to higher brain areas depends on the general state of arousal as well as on the activity of the whisker motor cortex, and the context of the animal’s environment. The sensory and motor systems of the whiskers are coupled by a number of sensorimotor feedback loops, allowing the animals to adjust whisker movements to sensory input. Unfortunately, many of the brain regions involved in these feedback loops have received relatively little attention with respect to the whisker system. Hence, our knowledge on the relative importance of these areas and their connections is incomplete. Yet, based on the current data available to us, we present a scheme of the relevant anatomical connections in Figure 8. Although these brain structures have many more connections, we have attempted to highlight the most prominent ones. However, the complexity of the whisker system seems to depend on the behavioral state; the more active an animal is, the more complex its whisker movements are and therefore a greater level of detail results during sensory and motor information processing.

ACKNOWLEDGMENTS
The authors thank Drs. Frank Rice and Hans Dringenberg for their comments on a previous version of the manuscript and Dr. Reha Erzurumlu for contributing an unpublished photomicrograph. We kindly thank the Netherlands Organization for Scientific Research (NWO; AH), the Dutch Organization for Medical Sciences (ZonMW; CIDZ), Life Sciences (ALW; CIDZ), Senter (Neuro-Baik; CIDZ), Primes Beatrice Fonds (CIDZ), and the SENSOPAC, CEREBNET, and C7 programs of the European Community (CIDZ) for their financial support. We also thank our lab members for valuable discussions.

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Chapter 1

Bozman et al.  

Anatomy of the whisker system


Bosman et al. Anatomy of the whisker system

Frontiers in Integrative Neuroscience

October 2011 | Volume 5 | Article 53 | 23

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Chapter 1

General Introduction

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Chapter 1


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Chapter 1

Boosan et al.

Anatomy of the whisker system


1.6 Scope of thesis

This thesis focuses on two brain regions, the cerebellum and the hippocampus, two regions that integrate sensory information for learning. The scientific method of asking a question, stating a hypothesis, and then testing that hypothesis by manipulating a variable while keeping all other things controlled can be addressed from many levels in neuroscience. Knockout mice, for example, have a targeted gene deleted or reduced. Manipulating genes have an effect on proteins thereby causing molecular differences, which can exhibit neurometric differences in targeted neurons, and therefore behavior.

In estimating psychometric sensitivity one can ask the subject: is the stimulus present (stimulus detection), are two stimuli perceived to be different (stimulus discrimination), is the stimulus a specific one in a group (identification) or does the stimulus belong to a specific category (categorization) [110]. Chapters 2.1, 2.2, and 3.2 use different forms of a discrimination task in order to control and investigate variables of behavior and in the case of chapter 2.2 how they relate to neurophysiology.

Neurometric differences may then have an effect on one or several brain regions where a specific gene is expressed causing differences in many systems which then may exhibit some behavioral phenotype. Conversely, one can manipulate behavioral parameters that show differences in neuronal responses and may even have permanent effects at the molecular level, for example vis a vis plasticity. So these macro to micro levels of manipulation (or vice versa) affect the brain in a fluid way, and these are tools for neuroscientists to attack a question from many viewpoints. Figure 1.6A illustrates different levels of investigation used in this thesis. An example of the interaction of these levels of analysis, with potential outcomes as discussed in Chapter 3.2, is also included. While there are always advantages and disadvantages of each technique, it is the combination of all of them that allow us to understand the brain.

This thesis aims to uncover how brain structures and their physiological representations of sensory stimuli are encoded, unitized and distinguished. Chapter 2.1 utilizes the rodent’s keen sense of olfaction and somatosensation showing that the hippocampus is necessary for recollection but not familiarity of previously learned, odor-medium paired associations. Chapter 2.2 demonstrates how CA1 hippocampal neurons encode prospective and retrospective trajectories of a learned, continuous alternation path, but rarely encode trajectory during a discrete trial conditional discrimination task dependent upon tactile-visual cues. Chapter 3.1 describes various whisker related encoding characteristics in the cerebellum such as differential complex spike and simple spike responses to whisker stimulation and spiking ensemble orientations related to cerebellar functional anatomy. This research was necessary in order to understand cerebellar representations of the whisker system for future whisker-based discrimination tasks. In continuation with this preliminary work, the role
of inhibitory input on purkinje cell activity are discussed within the framework of whisker stimulation in the awake mouse in chapter 3.2. The role of cerebellar plasticity in a whisker based object detection task is discussed in chapter 3.3.

This thesis does not focus on one brain region neither does it focus on one neural mechanism as is often done in a PhD thesis. This thesis focuses on, behavioral and neural coding properties in two regions required for different types of learning. It attempts to describe useful methods for uncovering systems level, physiological correlates of behavior for building a career investigating learning. Neuroscience utilizes a reductionist approach necessary for empirical inquiry. Science builds upon each discovery and allows for individual (micro) and field specific (macro) “learning” about the brain. Therefore, this thesis aims to gather pieces of knowledge in order to learn about learning.

Figure 1.6: This thesis aims to investigate the brain at many different levels. Both top down and bottom up approaches have been utilized to manipulate dependent variables, which ultimately have many effects. The green areas are variables for manipulation while the pink area represents aspects of investigation. Gray arrows show interrelatedness among levels while the red arrow represent a bottom up approach and the green arrow represents a top down approach. Small print at bottom and top of box are examples of possible effects.
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CHAPTER II

Hippocampal Processing and Multisensory Discrimination
A major controversy in memory research concerns whether recognition is subdivided into distinct cognitive mechanisms of recollection and familiarity that are supported by different neural substrates. Here we developed a new associative recognition protocol for rats that enabled us to show that recollection is reduced, whereas familiarity is increased following hippocampal damage. These results provide strong evidence that these processes are qualitatively different and that the hippocampus supports recollection and not familiarity.

BRIEF COMMUNICATIONS

Recognition memory: opposite effects of hippocampal damage on recollection and familiarity

Magdalena M Sauvage, Norbert J Fortin, Cullen B Owens, Andrew P Yonelinas & Howard Eichenbaum

A major controversy in memory research concerns whether recognition is subdivided into distinct cognitive mechanisms of recollection and familiarity that are supported by different neural substrates. Here we developed a new associative recognition protocol for rats that enabled us to show that recollection is reduced, whereas familiarity is increased following hippocampal damage. These results provide strong evidence that these processes are qualitatively different and that the hippocampus supports recollection and not familiarity.

Some of the most compelling data on recognition memory and hippocampal function involve the use of signal detection analyses. In these analyses, subjects are initially presented with a stimulus list and are then required to identify test stimuli as the same (old) items or different (new) stimuli across a range of confidence levels or response biases. In normal human subjects, the receiver operating characteristic (ROC) function for lists of single items is typically asymmetrical (reflecting an above-zero y intercept), interpreted by some to reflect a threshold for recollection, and has a curvilinear shape, reflecting the strength of familiarity (the dual process model). A major alternative view is that recognition is supported by qualitatively similar memory signals, wherein for each degree of curvilinearity reflects the sum of the strengths of memory components and the asymmetry reflects greater variability in strength for old than for new items (the unequal variance model). According to this latter view, familiarity and recollection differ only in sensitivity, such that familiarity reflects the detection of weaker memories, whereas recollection is experienced when memories are stronger or involve more information.

There is also compelling evidence indicating that recollection and familiarity may have distinct neural substrates, but the question of whether specific brain areas make qualitatively different contributions to recognition memory remains controversial. Evidence from studies on amnesia in humans have contributed to, but not resolved, these controversies. Amnesia consequent to transient hypoxia associated with hippocampal damage results in a decrease in the asymmetry of the ROC function, reflecting a deficit in recollection, but the curvilinear shape is relatively spared, indicating that there is no effect on familiarity, whereas damage that reaches into the parahippocampal region results in deficits in both recollection and familiarity. Our own ROC analyses, using an animal model where we definitively limited the damage to the hippocampus, resulted in a selective deficit in the same index of recollection (loss of asymmetry) and no impairment in familiarity (retained curvilinearity). However, deficits in both the asymmetry and curvilinearity of the ROC are also reported in amnesic patients with damage that is described as being limited to the hippocampus. A similar controversy exists over the findings from functional imaging studies. Different studies have shown either recollection-specific activation of the hippocampus or activation that occurs more generally in medial temporal lobe areas associated with the strength of memory.

A major difficulty in resolving the controversies concerning whether recollection and familiarity are qualitatively different processes and whether the hippocampus has a selective role is that the critical comparisons in the studies on both humans and animals rely on quantitative differences in memory performance or neural activation. Recent experiments, however, have suggested a way in which recollection and familiarity might be put into competition and, consequently, could be affected in opposite directions by hippocampal damage. These studies focus on associative recognition, an experimental protocol in which the subjects are initially presented with a list of stimulus pairs and must distinguish the previously experienced (old) stimulus pairings from rearranged (new) pairs of the same stimulus elements. When the pairs are processed as separate stimulus elements, performance may depend largely on recollection of the acquired associations, as old and new pairs cannot be distinguished on the basis of differential familiarity for the individual elements. Alternatively, when the elements of a pair are readily ‘unitized’ into a single configuration, such as when the elements are features of a face or parts of a compound word, familiarity can support memory for stimulus pairings just as it does for single stimuli. Both kinds of processing can contribute to recognition, and here we asked whether selective experimental damage to the hippocampus would decrease the contribution of recollection, consistent with a diminished ability to associate the elements, and conversely increase the contribution of familiarity, consistent with an uncovering of the ability of other brain areas to unitize the stimuli.

We developed a version of the associative recognition protocol for rats, using stimulus pairs composed of combinations of an ordinary household odor (for example, lemon, thyme and cumin) mixed into a digging medium (for example, wood chips, beads and sand) contained in a cup. Rats can readily learn to separately attend to odors and media as distinct stimulus dimensions, so we expected that substantial experience during initial training and testing with many combinations of the same stimulus elements would encourage the rats to distinguish these elements and rely on recollection of their associations (for example, lemon is associated with wood chips). Alternatively, odors and media could readily be unitized into scented medium configurations (for example, lemon-smelling wood chips), allowing the use of familiarity to make recognition judgments.
would be supported by observing qualitatively similar effects on both recollection and familiarity, or whether the single process model observing qualitatively distinct effects of selective hippocampal damage would determine whether the dual process model would be confirmed by observing performance largely on the basis of recollection in control rats and diminished recollection with enhanced familiarity in rats with selective hippocampal damage. The model analysis indicated that control rats relied mainly on recollection to solve tasks, as shown by a positive index of recollection $Ro$ ($y$-intercept $= 0.49$, Fig. 2a) and the absence of curvilinearity (familiarity index $d' = 0.0$, Fig. 2a), indicating that there was no contribution from familiarity. Characterization of the ROC function as linear was confirmed by observation of a curvilinear ROC function when the data were $z$-transformed ($z$-ROC quadratic coefficient significantly different from 0; $t_{z} = 3.792, P = 0.009$, Fig. 2b). Hippocampal damage reduced performance on the basis of recollection, reflected in a significant reduction of the recollection index $Ro$ ($y$-intercept $= 0.17, t_{z} = 4.47, P = 0.003$, Fig. 2a,c). In addition, and in marked contrast to control subjects, the ROC function for animals with hippocampal damage was curvilinear, as confirmed by an above-zero familiarity index ($d' = 0.55$, Fig. 2a,c) and a linear $z$-ROC function (quadratic coefficient not different from 0; $t_{z} = 1.385, P = 0.209$, Fig. 2b). Furthermore, a direct between-group comparison of $d'$ and $Ro$ scores (see Supplementary Methods) confirmed that damage to the hippocampus impaired recollection primarily, as indicated by an asymmetrical and linear ROC function. Rats with hippocampal damage were impaired in recollection and showed enhanced familiarity, reflected in a decrease in the $y$ intercept and the appearance of a curvilinear ROC function ($\pm$ s.e.m.). (b) The linearity of the ROC function for control subjects was confirmed by observation of a curvilinear function in $z$-space. Conversely, the curvilinearity of the ROC function for rats with hippocampal damage was confirmed by a linear $z$-ROC function. (c) Hippocampal damage had opposite effects on indices of recollection ($Ro$) and familiarity ($d'$). Bars represent means; $0 = $ individual score; $P$ values for the main effects and interaction are indicated across indices. Controls, sham-operated animals; hippocampus, rats with hippocampal damage.
Hippocampal Processing and Multisensory Discrimination

BRIEF COMMUNICATIONS

hippocampus had statistically significant opposite effects on familiarity and recollection (interaction: \( F_{3, 36} = 25.92, P = 0.001; \) post hoc \( t \), \( P = 0.027; \) Reo, \( P = 0.003 \)). Taken together, these results indicate that hippocampal damage produces a deficit in recollection and a complementary enhancement of familiarity. These findings, indicating qualitatively different recognition strategies, are all the more notable considering that overall performance, measured by the percent correct across all bias levels, did not significantly differ between the groups (sham 68 ± 2% and hippocampal 62 ± 1%, \( r_{13} = 1.14, P = 0.28 \)).

We then assessed the extent to which the unequal variance model could also fit the data, and we compared the predictions of both models using a model-independent regression analysis. Consistent with a large body of literature\(^\text{1-3}\), a goodness of fit analysis showed that the unequal variance model fit the behavioral data well (\( x^2_{\text{control}} = 0.29 \) and \( x^2_{\text{hippocampus}} = 0.44 \), where \( P < 0.05 \) requires \( x^2(4) \geq 9.49 \)), and indeed revealed a deficit in rats with hippocampal damage (\( r_{13} = 3.01, P = 0.010 \)). Notably though, the dual process model provided a slightly better fit (\( x^2_{\text{control}} = 0.19 \) and \( x^2_{\text{hippocampus}} = 0.34 \)). Moreover, direct comparisons using model-independent linear and quadratic regression analyses supported the predictions of the dual process model and not those of the unequal variance model. The ROC function of control subjects was linear (no significant alteration of the curve by adding a quadratic component, \( R^2_{\text{quad}} \) compared with \( R^2_{\text{lin}} \), \( t_6 = 1.36, P = 0.225 \); Supplementary Fig. 1 online). In contrast, the ROC function of rats with hippocampal damage was curvilinear, as confirmed by a significant alteration of the function by adding a quadratic component to the equation (\( R^2_{\text{quad}} \) compared with \( R^2_{\text{lin}} \), \( t_6 = 0.05 \)). The observation of a linear ROC is inconsistent with the unequal variance model. Furthermore, this model cannot account for the opposite effects of hippocampal damage on the curvilinearity (higher than controls) and \( y \) intercept (lower) of the regressions. In contrast, this combination of findings is fully consistent with the predictions of the dual process model\(^\text{1-2}\).

These results provide the first evidence that rats, like humans, can rely primarily on recollection in associative recognition. The findings also show that control rats and rats with hippocampal damage can perform at a similar overall level on an associative-recognition task by using different strategies, with the control subjects relying mainly on familiarity, and consequently reduce the deficit in amnesic patients that is normally observed in associative recognition\(^\text{13}\). Also, recent studies have shown that hippocampal damage increases the tendency to unitize stimulus elements into configural stimuli\(^\text{14}\). For example, rats with hippocampal damage tend to unitize pairs of odor stimuli that are presented in close juxtaposition in simultaneous discrimination problems, and they subsequently perform poorly when required to identify individual stimuli selected from different pairs. Also, in monkeys, the explicit learning of visual stimulus configurations is facilitated over that of normal animals by damage limited to the hippocampus, whereas configural representation is severely impaired following damage to the perirhinal cortex\(^\text{13}\). Consistent with these findings, we suggest that rats with hippocampal damage and preserved perirhinal function have an increased tendency to unitize the elements of stimulus pairs, allowing them to employ familiarity as a compensatory strategy for distinguishing new and old pairs.

The present results provide the first evidence that recollection and familiarity are qualitatively dissociable and distinctively affected by hippocampal damage. The pattern of opposite effects on recollection and familiarity cannot be explained by models in which recollection and familiarity involve qualitatively similar processes contributing to a continuous memory strength signal\(^\text{2-9}\). Furthermore, these results are inconsistent with the view that the hippocampus supports both recollection and familiarity. Instead, these findings provide compelling evidence that the hippocampus and other areas (such as the perirhinal cortex) make distinct and complementary contributions to memory.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We thank C. Ergold, L. Devito and N. Simures for help with behavioral testing, and L. Ho and S. Hutton for assistance with histological processing. This work was supported by US National Institute of Mental Health grants MH52090 and MH17102.

AUTHOR CONTRIBUTIONS

M.M.S. designed and conducted the experiment and data analyses, and wrote the manuscript. N.J.F. consulted on data analyses, and A.P.Y. consulted on manuscript preparation. C.B.O. participated in conducting the experiment. H.E. supervised the project and participated in writing the manuscript.

Published online at http://www.nature.com/natureneuroscience

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Supplementary figure. Regression fits. Linear (line) and quadratic (dash) regression lines are fitted to the data a. Control subjects’ data are well fit by a linear regression and the fit is not improved by adding a quadratic component b. The regression on data from rats with hippocampal damage benefit by addition of a quadratic component. c. For all comparisons of linear and quadratic regressions, the Y-intercept (+ SEM) is higher in control subjects than for rats with hippocampal damage.

Recognition memory: Opposite effects of hippocampal damage on recollection and familiarity

Magdalena M. Sauvage, Norbert J. Fortin, Cullen B. Owens, Andrew P. Yonelinas and Howard Eichenbaum

Supplementary material:

Methods

Adult Long Evans male rats (225-250g) were maintained under reverse light/dark cycle (6 a.m. light off, 6 p.m. light on), food deprived to 85% of their body weight and received water ad libitum. Testing took place during the day in the home cage. During shaping, rats learned to retrieve 1/4 Froot Loop cereal buried in each unscented medium contained in a 125 ml plastic cup (NALGENE). Subsequently, a pool of eleven media and ten odors was used, allowing the creation of 110 distinct odor-medium pairings. Ten original (old) and ten rearranged (new) pairs of odor-medium were chosen randomly for each daily session (5 sessions/week). Rats were trained on a delay non-matching to sample task in which a single stimulus pair baited with 1/4 Froot Loop was presented, then after a 1 min delay, the old pair and a new pair were presented sequentially. To ensure that rats could not solve the task using olfactory cues, both cups were baited but the bait in the original pair was made inaccessible. As animals acquired the task (80% correct over 3 consecutive 10 trial sessions), the number of original pairs increased from 1 to 10 and the delay between initial presentations and testing increased from 1 min to 30 min. Rats were trained to the same criterion then over-trained for
10 additional sessions. Subsequently, 5 response criteria were generated by varying the size of the cup and the amount of reward. Once probabilities for hit and false alarms were within a range of 0.2 for each bias over three consecutive sessions, data were collected for 2 additional sessions for each bias and subjects were divided into two groups of equivalent performance in d’ and Ro and surgeries for sham or hippocampal lesions were performed. Following two weeks of recovery, rats received six testing sessions per bias and the five last sessions were averaged and used to construct the ROC curves (fig. 2a).

\( R^2 \) quadr and \( R^2 \) lin were obtained by fitting polynomial and linear regression lines to each individual data set. Unpaired t-tests were used to compare Ro, d (fig. 2c) and percent correct scores. To directly compare scores on recollection and familiarity, Ro and d scores of each subject were normalized as standard deviations from the average for the same variable and a two-way ANOVA was performed with Ro and d’ as parameters followed by unpaired t-tests.

**Generating ROC and z-transformed ROC functions, Ro and d’ estimates**

ROC functions were generated for each animal by plotting the hit rate against false alarm rate in each response bias condition. ROCs were quantified further by taking the z-scores of the hits and false alarms and plotting the ROCs in z-space. A least squares algorithm (Yonelinas et al., 1997) was used to estimate the intercept and the degree of curvilinearity observed in each ROC, which reflects recollection (Ro) and familiarity (d’), respectively. Because lure items could be recollected as having been recombined, an additional recollection parameter was included for the lure items (Rn), but this parameter was close to zero and is not discussed further. The model equation, \( P(\text{old}|\text{old}) = P(\text{old}|\text{new}) + Ro + (1-Ro)\phi(d'/2-c) - (1-Rn)\phi(-d'/2-c) \), assumes that recognition reflects the contribution of recollection process and a signal detection based familiarity process. The variable d’ reflects the distance between two equal-variance Gaussian strength distributions, c, reflects the response criterion at point i, and \( \phi \) is the cumulative normal response function.

*Yonelinas AP. Mem Cognit. 25 (6):747-63 (1997)*
Spatial representations in dorsal hippocampal neurons during a tactile visual conditional discrimination task

Trajectory-dependent coding in dorsal CA1 of hippocampus has been evident in various spatial memory tasks aiming to model episodic memory. Hippocampal neurons are considered to be trajectory-dependent if the neuron has a place field located on an overlapping segment of two trajectories and exhibits a reliable difference in firing rate between the two trajectories. It is unclear whether trajectory-dependent coding in hippocampus is a mechanism used by the rat to solve spatial memory tasks. A first step in answering this question is to compare results between studies using tasks that require spatial working memory and those that do not. We recorded single units from dorsal CA1 of hippocampus during performance of a discrete-trial, tactile-visual conditional discrimination (CD) task in a T-maze. In this task, removable floor inserts that differ in texture and appearance cue the rat to visit either the left or right goal arm to receive a food reward. Our goal was to assess whether trajectory coding would be evident in the CD task. Our results show that trajectory coding was rare in the CD task, with only 12 of 71 cells with place fields on the maze stem showing a significant firing rate difference between left and right trials. For comparison, we recorded from dorsal CA1 during the acquisition and performance of a continuous spatial alternation task identical to that used in previous studies and found a proportion of trajectory coding neurons similar to what has been previously reported. Our data suggest that trajectory coding is not a universal mechanism used by the hippocampus to disambiguate similar trajectories, and instead may be more likely to appear in tasks that require the animal to retrieve information about a past trajectory, particularly in tasks that are continuous rather than discrete in nature.
Spatial Representations in Dorsal Hippocampal Neurons During a Tactile-Visual Conditional Discrimination Task

Amy L. Griffin,* Cullen B. Owens, Gregory J. Peters, Peter C. Adelman, and Kathryn M. Cline

ABSTRACT: Trajectory-dependent coding in dorsal CA1 of hippocampus has been evident in various spatial memory tasks aiming to model episodic memory. Hippocampal neurons are considered to be trajectory-dependent if the neuron has a place field located on an overlapping segment of two trajectories and exhibits a reliable difference in firing rate between the two trajectories. It is unclear whether trajectory-dependent coding in hippocampus is a mechanism used by the rat to solve spatial memory tasks. A first step in answering this question is to compare results between studies using tasks that require spatial working memory and those that do not. We recorded single units from dorsal CA1 of hippocampus during performance of a discrete-trial, tactile-visual conditional discrimination (CD) task in a T-maze. In this task, removable floor inserts that differ in texture and appearance cue the rat to visit either the left or right goal arm to receive a food reward. Our goal was to assess whether trajectory coding would be evident in the CD task. Our results show that trajectory coding was rare in the CD task, with only 12 of 71 cells with place fields on the maze stem showing a significant firing rate difference between left and right trials. For comparison, we recorded from dorsal CA1 during the acquisition and performance of a continuous spatial alternation task identical to that used in previous studies and found a proportion of trajectory coding neurons similar to what has been previously reported. Our data suggest that trajectory coding is not a universal mechanism used by the hippocampus to disambiguate similar trajectories, and instead may be more likely to appear in tasks that require the animal to retrieve information about a past trajectory, particularly in tasks that are continuous rather than discrete in nature. © 2010 Wiley Periodicals, Inc.

KEY WORDS: disambiguation; CA1; local cue; single unit; trajectory coding

INTRODUCTION

One of the most striking features of hippocampal neurons is their ability to reliably and robustly code location (O’Keefe and Nadel, 1978). In recent years, there have been several studies that have demonstrated that, in addition to this spatial code, hippocampal neurons also code specific trajectories by exhibiting different firing rates depending on the previous or upcoming location. In the first study to demonstrate trajectory coding, Wood et al. (2000) showed that two-thirds of hippocampal neurons that had place fields on the central stem of a T-maze during a continuous spatial alternation (SA) task showed significant differential firing rates between left- and right-bound trials. A similar finding was reported for rats performing a hippocampus-dependent serial reversal task in a plus maze (Ferbinskianu and Shapiro, 2003). In this study, 59% of the hippocampal neurons recorded were “journey-dependent,” with significantly greater proportion of these neurons showing retrospective coding (showing differential firing rates depending on a prior spatial location) than prospective coding (showing differential firing rates depending on an upcoming location). Subsequently, there have been a number of studies using a variety of spatial tasks that have demonstrated that hippocampal neurons code contextual variables (Ainge et al., 2007a; Frank et al., 2000; Lee et al., 2006; Lippton et al., 2007; Smith and Mizumori, 2006; but see Lenck-Santini et al., 2001). Bower et al. (2005) observed that hippocampal neurons exhibit trajectory coding during a sequence disambiguation task depending on the task parameters. Trajectory coding was seen in the “skipped reward” task in which the rat ran continuously from the beginning to the end of the sequence without pausing for reward and the “barrier trained” version of the task in which the rats were pretrained on the task using barriers to guide their movements. Trajectory coding was not seen, however, in the “complex sequence” task in which the rat paused for rewards at the end of each segment of the sequence. It remains unclear why trajectory coding appears in some tasks and not in others and what the coding might mean when it does appear. A related computational model showed that trajectory coding could serve as a memory retrieval signal used to guide the rat to the appropriate goal (Hasselmo and Eichenbaum, 2005). If this is true, trajectory coding would be more common in tasks that require the animal to remember a past event or action and should be rare in tasks that do not require working memory.

In this study, we recorded CA1 hippocampal neurons during performance of a tactile-visual conditional discrimination (CD) task. On each trial, the maze floor is covered with a textured insert (light brown wood or black plastic mesh) that signals which goal arm will contain food reward. Importantly, unlike SA, this task does not rely on the memory of a previous
goal location to make a correct choice. Instead, the task relies on the ability to form a relationship between local cues on the maze and a specific goal arm choice. The formation of this type of configurational relationship between a spatial location and local cues has been suggested to depend on the hippocampus (Hirsh, 1974; Rudy and Sutherland, 1995; Sutherland and Rudy, 1989). In fact, the acquisition of this type of visual-spatial CD was shown to be disrupted by hippocampal damage induced by ischemia (Modo et al., 2000) and by excitotoxic lesions of the dorsal hippocampus (Murray and Ridley, 1999).

Place cells are known to exhibit remapping when a familiar environment changes. Local or proximal cues such as those used in this study have been shown to drive place cell firing for a subset of recorded hippocampal neurons (Knierim, 2002; Shapiro et al., 1997). If place cells are driven by local cues in tasks that do not require a specific behavioral response, we reasoned that it was likely that place cells would be even more likely to code local cues that guide the rat’s decision about which goal arm to visit. Therefore, we sought to investigate whether trajectory-dependent coding would be evident in the tactile-visual CD task.

### MATERIALS AND METHODS

#### Animals and Surgery

Six, male, Long–Evans hooded rats (weighing 400–450 g) were individually housed and maintained on a 12:12-h light/dark cycle in a temperature and humidity-controlled colony room with *ad libitum* access to food and water. During the period of behavioral training and recording, rats were maintained at 90% of their free-feeding body weight. For implantation of the recording microdrive, rats were given a pre-anesthetic dose of atropine (0.05 mg/kg) and anesthetized with isoflurane (1.5–3% in oxygen). The skull surface was exposed and cleaned. Six to seven small holes were drilled near the skull ridge using a stereotaxic-mounted drill (Fine Science Tools). Five to six anchor screws (4.0 mm long, shaft diameter 0.85 mm) and one ground screw (located above the cerebellum) were affixed to the skull with dental acrylic. A circular hole was then drilled for the recording microdrive, rats were given a pre-anesthetic dose of atropine (0.05 mg/kg) and anesthetized with isoflurane (1.5–3% in oxygen). The skull surface was exposed and cleaned. Six to seven small holes were drilled near the skull ridge using a stereotaxic-mounted drill (Fine Science Tools). Five to six anchor screws (4.0 mm long, shaft diameter 0.85 mm) and one ground screw (located above the cerebellum) were affixed to the skull with dental acrylic. A circular hole was then drilled above dorsal hippocampus (4.0 posterior to bregma, 2.0 lateral to bregma) using a 2.7-mm-diameter trephine (Fine Science Tools). The dura was removed, and the brain surface was kept moist using gelfoam soaked in sterile saline. A microdrive containing 16 tetrodes (composed of four 12.7-μm-diameter nichrome wires, 150–300 kΩms at 1 kHz in gold solution), four reference wires, and a ground wire was carefully lowered onto the brain surface. After the microdrive was fixed to the skull with dental acrylic, each tetrode and reference wire was immediately advanced 1.13 mm into the brain. Rats were given Children’s ibuprofen (30 mg/kg) in their drinking water for 2 days for pain relief and allowed to recover for at least 1 week before behavioral testing and recording began. All procedures were approved by the University of Delaware Institutional Animal Care and Use Committee.

#### Tetrode Adjustment and Recording

After the rats recovered from surgery, the reference wires were advanced until they were positioned just dorsal to the CA1 layer of dorsal hippocampus (picking up distant sharp waves but no ripple (Buzsaki, 1986; Chrobak and Buzsaki, 1996; Fig. 2). The tetrodes were then lowered gradually over the course of 7–10 days until they reached stratum pyramidale, as evidenced by sharp waves, accompanied by large-amplitude ripples in the local field potential record and units with at least a 3:1 signal-noise ratio. Tetrode adjustments were made as the rats rested on a rotating pedestal located in the corner of the recording room. This pedestal could be rotated to untangle the tether if necessary. The rats’ position data were recorded from a camera mounted above the maze that captured luminance (30 Hz) emitted from an array of light-emitting diodes located on the rat’s headstage. Position data, units, and field potentials were recorded using a 64-channel digital recording system (Digital Lynx; Neuralynx, Bozeman, MT).

#### Behavioral Training

The behavioral tasks were conducted on a T-maze constructed of wood and painted black. The maze consisted of a central stem (116 × 10 cm), two goal arms (56.5 × 10 cm, each), and two return arms (112 × 10 cm) (Fig. 1A). A small plastic cup was located at the end of each goal arm for delivery of the chocolate sprinkle reward. There was a stationary intertrial interval (ITI) pedestal (different from the rotating pedestal used for tetrode adjustments) located near the base of the maze stem, where the rat waited between trials. The testing room was illuminated by a single compact florescent bulb and surrounded by black curtains with large visual cues attached. Before surgery, each rat was handled by the experimenter and acclimated to the testing room and maze. Five of the rats were trained on the CD task until they reached the criterion of maintaining above 80% choice accuracy for two consecutive days. Figure 1A shows a schematic of the CD task. Before each trial, wooden inserts covered on one side by black plastic mesh were placed on the floor of the maze stem and goal arms with either the bare wood or mesh side facing up. Rats were rewarded for making either a right or left turn, depending on the texture of the floor insert. Two of the rats were required to learn the “right on mesh, left on wood” rule, and the remaining three rats learned the “left on mesh, right on wood” rule. The ITI was 8–12 s, during which the rat was confined to the ITI pedestal using a large wooden blocker while the experimenter set up the next trial. To ensure that the rat did not anticipate the upcoming trial during the ITI by using auditory cues, the experimenter flipped the maze inserts during each ITI, even between two consecutive identical trial types. Rats were given 24 trials per day (12 wood/12 mesh) in a pseudorandom sequence (Fellows, 1967). After recovery from surgery, each rat was given at least 3 days of training on the CD task to make sure that choice accuracy was at presurgery levels before recording began. One rat was assigned to perform the continu-
ous SA task instead of the CD task. For this rat, training procedures were identical to methods used previously (Lee et al., 2006). Briefly, the rat was pretrained to perform left-only traversals on the maze, and then on the first day of recording, the rat performed 15 left laps (right goal arm blocked), 15 right laps (left goal arm blocked), and then all blockades were removed, and the rat was rewarded for alternating between the right and left goal arm. This rat performed 80–100 trials per day for 16 days of recording. The recording sessions were kept similar in duration between the two tasks. This resulted in there being more trials in the SA task (~100) than the CD task (24) because of the ITI and discrete nature of the CD task.

Histology
At the conclusion of the experiment, marking lesions were made by passing 10 μA of current through one wire of each tetrode and the reference wires. After 24 h, rats were then perfused with 0.9% saline, followed by 4% formalin, and the head was soaked in formalin for 2–3 days. After raising the tetrodes out of the brain, the brain was removed from the skull.
and placed in formalin and transferred to sucrose solution. After sinking, the brains were frozen and sectioned (40 μm). The sections were mounted on slides and stained using cresyl violet. Tetrode tracks were reconstructed using the tetrode adjustment record and by visualizing the sections under a microscope.

**Data Analysis**

Clusters were isolated offline using commercially available software (SpikeSort 3D; Neuralynx, Inc., Bozeman, MT). Waveform profiles of units were compared across sessions to ensure that the same unit was not included in the analysis more than once. Pyramidal cells were distinguished from interneurons based on an average firing rate below 2 Hz and a spike duration greater than 0.3 ms (Ranck, 1973). A custom MATLAB script used the position data to assign timestamps to the beginning and end of each trial. Correct trials were divided into right and left trial types. For both right and left trial types, the position data points were divided into 55 5-cm bins covering the stem, goal arm, and return arm (Fig. 1B), and total occupancy was calculated for each bin (Lee et al., 2006). For each cluster, spikes were assigned to a position timestamp and x-y coordinate using a custom MATLAB script. Place fields were defined as described previously (Lee et al., 2006): four or more adjacent bins with a firing rate of at least 10% of the peak firing rate for that cell. For cells with a firing field on the stem of the maze, firing rate distributions were then calculated separately for right and left-turn trials by dividing the number of spikes emitted while the rat occupied each bin by the time spent in each bin. The firing rates were averaged across all stem bins, yielding one data point for each trial. Then, stem firing rates on left and right trials were compared using a t-test. Cells were considered to be trajectory-dependent if the average stem firing rate was significantly different between right and left trials. The procedure for constructing the firing rate histograms, including binning the maze and comparing firing rates on left and right trials, was identical between the CD rats and the SA rat.

To analyze the activity of cells during the ITI, peri-event time histograms were constructed using a custom MATLAB program. The peri-event time histograms were triggered by the completion of each trial, signaled by the rat leaving either the right or left return arm and entering the ITI pedestal. The mean firing rate during the first 30 s of the ITI was then calculated for each trial. If the mean firing rate was above 1 Hz, firing rates were compared between left and right trials using a t-test. Because of inadequate numbers of error trials, only correct trials were analyzed.

**RESULTS**

**Histology**

Figure 2 shows a representative histological section from a rat that performed the CD task. The anterior–posterior and medi–lateral placement of the recording microdrives were similar between all six rats, suggesting that pyramidal cells were recorded from similar locations along the septo-temporal and proximal–distal axis of CA1 in all rats.

**Behavioral Results**

Rats learned the tactile–visual spatial CD (performed at or above 80% correct for two consecutive days) in 6–11 days (M = 7.75, standard deviation (SD) = 2.22; Fig. 1D). Once the CD task was learned, rats maintained a high level of accuracy (above 80% correct) throughout the subsequent testing/recording sessions. The rat that was trained on continuous alternation rapidly acquired the task, performing at or above 80% correct from the fifth training session onward (data not shown).

**Electrophysiological Results**

We recorded a total number of 531 pyramidal cells, 415 in the CD task (five rats) and 116 in the continuous alternation task (one rat). As seen in Table 1, the total number of cells included in the analysis was distributed across the six rats, ranging from 34 to 145 cells included from an individual rat. Of
TABLE 1.

<table>
<thead>
<tr>
<th>Number of cells recorded</th>
<th>Number of stem-firing cells</th>
<th>Number of trajectory-coding cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 1–CD</td>
<td>38</td>
<td>5</td>
</tr>
<tr>
<td>Rat 2–CD</td>
<td>145</td>
<td>27</td>
</tr>
<tr>
<td>Rat 3–CD</td>
<td>141</td>
<td>23</td>
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<tr>
<td>Rat 4–CD</td>
<td>57</td>
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</tr>
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<td>1</td>
</tr>
<tr>
<td>Rat 6–CA</td>
<td>116</td>
<td>24</td>
</tr>
</tbody>
</table>

CD, conditional discrimination; CA, continuous alternation.

the cells recorded during the continuous alternation task, 24 (21%) had firing fields on the central stem of the T-maze. Similarly, of the cells recorded during the CD task, 71 (17.1%) had firing fields on the maze stem. We observed only 12 of 71 (16.9%) cells that showed significant firing rate difference between left and right trial types in the CD task (Fig. 3 and Table 1). Of the 12 cells that showed differential firing, one third had fields restricted to the area approaching the choice point of the maze. The other two-thirds of cells had fields in various locations along the stem. In contrast, and consistent with previous findings (Wood et al., 2000), 14 of 24 (58%) units showed a significant firing rate difference between left and right trials in the continuous alternation task (Fig. 4). The proportion of trajectory coding units in this rat increased across days, with no stem-firing units being trajectory-dependent on an early session (Session 3; 6 units) and all stem-firing units showing trajectory-dependent firing thereafter (Sessions 13, 15, and 16; 12 units total). Performance during this early session was relatively poor: 64% correct, and performance improved thereafter, remaining above 80% correct on subsequent sessions. Figure 5 shows that there was a significantly larger proportion of trajectory coding units in the continuous alternation task than the CD task ($z^2(1) = 10.15, P = 0.001$).

To assess whether left and right trials were differentially coded during the ITI, we compared ITI firing rates following left and right trials for cells that showed elevated activity during the ITI, which we defined as having a firing rate during the ITI of 1 Hz or greater. Of the 415 units recorded, 75 units were included in the analysis. Of these cells, 10 (13.3%) showed a significant difference in firing rate depending on whether the rat had just made a right or a left turn. This result suggests that a subset of hippocampal neurons show retrospective coding in the CD task, coding the type of trial that had just been completed before the ITI.

To investigate whether subtle behavioral differences between the two tasks were responsible for the lack of trajectory coding in the CD task compared with the SA task, we compared the average lap duration and path deviation between the rat that performed SA and the five rats that performed CD. For both the path deviation and lap duration measures, the SA rat fell within the 95% confidence interval of the distribution of the CD rats, suggesting that the rat performing SA and the rats performing CD did not show overt differences in their trajectories that could account for the different proportions of trajectory-coding neurons.

We recorded from neurons in CA1 of dorsal hippocampus while rats performed a tactile-visual CD task. Our results show that trajectory coding was quite rare in the CD task, with most hippocampal neurons with firing fields on the stem of the T-maze firing similarly on left and right trials. To ensure that the lack of trajectory coding was not due to subtle differences in training procedures between our laboratory and other laboratories that have observed trajectory coding, we also recorded dorsal hippocampal CA1 neurons during the acquisition and performance of a continuous SA task. As shown in previous studies (Ferbinteanu and Shapiro, 2003; Frank et al., 2000; Lee et al., 2006; Lipton et al., 2007; Wood et al., 2000), during performance of the continuous SA task, over half of the neurons with firing fields on the maze stem showed a significant difference in firing rate depending on whether the rat was performing a left-turn or right-turn trial.

Our results are consistent with a recent study that found a lack of trajectory coding in a task that used motivational state (hunger or thirst) to signal the correct goal box choice (Kennedy and Shapiro, 2009). In this experiment, the three goal boxes were visually distinct and were moved to different positions on each trial. Therefore, the rats’ trajectory was determined both by the nonspatial contextual cue (motivational state) and a second nonspatial cue of the goal box appearance. Even though our study also used a nonspatial cue (floor inserts) to signal the appropriate goal box, this cue always signaled the same trajectory. Therefore, our results show that even when a nonspatial cue signals a specific trajectory, little trajectory coding is observed.

Although both the CD and SA tasks require a two-choice discrimination in a T-maze, there are several key differences between the CD and SA tasks that could have contributed to the difference in the proportion of trajectory coding neurons. Most importantly, there was a significant strategy difference between the tasks. The SA task requires the rat to remember the previously rewarded goal arm to alternate to the opposite goal arm. For the CD task, it is not necessary, and would in fact be detrimental to performance, for the rat to make a choice on the current trial based on the previously rewarded goal location. Because we presented the wood and mesh trials in a pseudorandom sequence, a rat using an alternation strategy would only be rewarded on ~50% of the trials. Instead, the CD task requires that the rat use cues present on the maze to guide its behavior. The absence of robust trajectory coding in

Hippocampus
FIGURE 3. Example CA1 pyramidal neurons recorded during the tactile-visual conditional discrimination task. Locations visited by the rat (blue traces) are superimposed with spikes that fired while the rat occupied that location on right-turn (red dots) and left-turn (green dots) trials. The histograms show the spatial firing rate distributions for right and left trials (red and green, respectively). Cell 6 was one of the two stem-firing neurons that exhibited a significant stem firing-rate difference between left and right trials. The mean stem firing rate is shown above each plot. The significance value of the $t$-test comparing firing rates on left and right trials and percentage of correct trials in the session are below the plots. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
FIGURE 4. Example CA1 pyramidal neurons recorded during the continuous spatial alternation task. As in Figure 3, the blue traces denote trajectories and red and green dots indicate spikes that fired on right and left trials, respectively. Unlike Figure 3, the beginning and ending of the trajectories were designated at the reward zones, where the rat paused to consume reward. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
the CD task compared with the SA task could be because the SA task requires the rat to keep a record of which goal arm was visited on the last trial to make a correct choice on the next trial. Other than the strategy difference between the tasks, trials on the CD task were separated by a brief ITI to allow the experimenter to set up the floor inserts for the next trial, whereas, in SA, the rat performed the task continuously, in a stereotypical “figure-8” pattern. Trajectory coding may be a mechanism that allows the rat to disambiguate similar trials and that this disambiguation signal becomes less necessary when the trials are discrete. It has been suggested that the trajectory-coding may be generated in hippocampus for hippocampus-dependent task such as serial reversals (Ainge et al., 2007a; Ferbinteanu and Shapiro, 2003; Smith and Mizumori, 2006) and that context-dependent activity reflects activity generated in structures upstream of the hippocampus in tasks that do not require the hippocampus (Ainge et al., 2008). An alternative possibility is that during SA, the populations of neurons are part of two separate reference frames, one that is bound to the left-to-right trajectory and another that is bound to the right-to-left trajectory, similar to directional place cells recorded on a linear track (Gothard et al., 1996; O'Keefe and Recce, 1993). Battaglia et al. (2004) recorded dorsal hippocampal neurons as rats shuttled back and forth on a circular track covered with multimodal local cues. This manipulation led to an increase in the proportion of neurons that fired in a bidirectional manner. Similar to our findings, the introduction of local cues caused the hippocampal network to code the spatial location regardless of differences in local cues. In this study, this was the case even when the local cues signaled the appropriate behavior that would lead to reward.

Although most reports of trajectory coding interpret the differential activity on left and right trials as a memory-related mechanism used by the animal to solve the continuous SA task, the direct link between trajectory coding and memory has yet to be established. Complete bilateral lesions of the hippocampus showed no disruption of performance of the SA task (Ainge et al., 2007b). Therefore, trajectory-dependent activity is presumably not necessary for task performance. Even more puzzling is that some tasks that do require the hippocampus, such as delayed alternation, do not yield trajectory-coding neurons. Ainge et al. (2007b) assessed both trajectory coding and the impact of complete bilateral hippocampal lesions on a delayed SA task. They showed that hippocampal lesions disrupted performance in well-trained rats and that trajectory coding disappeared when a short delay was given between trials (Ainge et al., 2007b). This same study also compared firing rates between left and right trials for cells with place fields in the start box and observed that a significant proportion of these cells showed trial-type-dependent firing. Similarly, Pastalkova et al. (2008) examined hippocampal neuronal activity during the delay period of a SA task and demonstrated that the population of recorded neurons fired in a unique sequence for left and right trials, with the sequence predicting the upcoming choice of the rat. In this study, we did a similar analysis wherein we compared firing rates in the ITI between left and right turn trials and found that approximately 13% of neurons that were active during the ITI showed significant differences in firing rates depending on the trial type just performed by the rat. Together, these findings suggest that the lack of differential activity on the maze stem may be because trial differences are coded while rats pause between trials. In a study using a delayed-nonmatch-to-position task, Griffin et al. (2007) observed context-dependent coding of hippocampal neurons when comparing sample (forced) trials and choice trials, but there were few neurons that showed robust firing rate differences between left and right choice trials. Another study showed trajectory coding in a sequence disambiguation task that is assumed to be hippocampus-dependent based on disruption of task performance when the room cues were rearranged (Bower et al., 2005). In interpreting Wood et al.’s (2000) findings, Bower et al. (2005) suggested that the method used to initially train the rats on the continuous SA task may have contributed to the robust trajectory coding seen during asymptotic performance. Specifically, Wood et al. (2000) used barriers on the initial days of training to shape the rats to perform the alternation task. Even though these barriers were removed at the time of recording, it was suggested that the long-term memory of the experience of the discriminative cues could have induced trajec-

FIGURE 5. The percentage of trajectory-coding neurons was significantly lower in the conditional discrimination task (top) than the continuous spatial alternation task (bottom; \( \chi^2(1) = 10.15, P = 0.001 \)).

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90
tory-dependent hippocampal activity. This suggestion was supported by the demonstration that hippocampal neurons showed robust context-dependent activity in rats initially trained using barriers to guide their movements toward the reward ports (Bower et al., 2005). Our results show, however, that even when discriminative cues (floor inserts that signal correct goal-arm choices) are present at the time of recording, the hippocampus does not show robust trajectory coding. It should be noted that trajectory coding was not seen in the Bower et al. (2005) study on a version of the task that required the rats to pause for reward between segments of the learned sequence ("the complex sequence task"). Thus, it seems that tasks that involve an interruption in trajectories, either by imposing a delay, an ITI, or a pause for reward are not as likely to show trajectory coding. Consistent with this idea, even the rat that performed SA in this study displayed few trajectory-coding neurons during early sessions, when the task was not well-learned. This could be explained by the fact that the rat paused more often during the early sessions, causing a significant delay between reward zone visits.

The fact that trajectory coding is seen during some tasks for which the hippocampus is not required and is not present in some hippocampus-dependent tasks is evidence against this coding being an essential signal for use in selecting an appropriate response. This study is yet another example of a lack of trajectory coding on a task that is likely to be dependent on the hippocampus (Modo et al., 2000; Murray and Redley, 1999). However, it should be noted that these two studies used a Y-maze instead of a T-maze and slightly different cues than used in this study. Future studies could examine the impact of hippocampal disruption on the acquisition and performance of the CD task using the same parameters as this study to unequivocally demonstrate whether or not the task is hippocampus-dependent. It is especially interesting that we observed so few trajectory coding neurons given the fact that our task involved presenting salient cues on the maze that signaled the correct behavioral choice. Given that hippocampal neurons have been shown to be sensitive to these types of local cues, it is quite surprising that the neurons represented the stem area similarly despite the difference in visual appearance and tactile stimulation of the two cues. It is possible that the initial acquisition of the CD task depends on the hippocampus, but that the maintenance of asymptotic performance relies on a separate system of brain structures. Future studies could (1) record hippocampal neurons during the acquisition period of the task to assess whether trajectory coding is more prominent during the acquisition of CD and (2) perform lesions or reversible inactivations during acquisition and performance of the CD task to delineate whether the hippocampus is crucial for task acquisition, performance, or both. Another future direction would be to record the same cells across the SA and CD task to investigate whether a trajectory-sensitive cell in SA would maintain its selectivity in CD or, conversely, whether the knowledge of the CD task would prevent trajectory-coding from developing in SA. Another future study could record from CA3 during the CD task. One alternative explanation for the current results is the notion that CA1 is responsible for temporal processing, which is an important component of the SA task. During SA, the rat must choose which goal arm to visit based on the immediately preceding trial without being distracted by earlier trials. Conversely, CA3 is thought to be important for associative coding that would be necessary during the CD task, in which the rat must link the texture and appearance of the floor insert with the correct goal arm (Rolls and Kesner, 2006; Kesner and Hunsaker, 2010). If CA3 is indeed responsible for associative coding, there should be a large proportion of CA3 neurons that discriminate between left and right trials during the CD task.

In summary, our results show that during a tactile-visual CD task that requires the rat to use local cues on the maze to make a behavioral choice, the majority of hippocampal neurons with place fields on the maze stem fired independently of trajectory. Conversely, hippocampal neurons showed robust trajectory-dependent coding in a continuous SA task that was conducted in the same room and on the same maze as the CD task, but requires knowledge of the previous trajectory for successful performance. Together with previous studies, these results suggest that trajectory coding may be more common in tasks that (1) impose a working memory demand and/or (2) require the rat to perform the task continuously, facilitating a need for reduction in trial-by-trial interference.

Acknowledgments

The authors thank James D’Amour for technical assistance and Mark Stanton for comments on an earlier version of the manuscript.

REFERENCES


CHAPTER III

Cerebellar Processing and Sensorimotor Integration
3.1

Encoding of whisker input by cerebellar Purkinje cells

The cerebellar cortex is crucial for sensorimotor integration. Sensorimotor inputs converge on cerebellar Purkinje cells via two afferent pathways: the climbing fibre pathway triggering complex spikes, and the mossy fibre–parallel fibre pathway, modulating the simple spike activities of Purkinje cells. We used, for the first time, the mouse whisker system as a model system to study the encoding of somatosensory input by Purkinje cells. We show that most Purkinje cells in ipsilateral crus 1 and crus 2 of awake mice respond to whisker stimulation with complex spike and/or simple spike responses. Single-whisker stimulation in anaesthetised mice revealed that the receptive fields of complex spike and simple spike responses were strikingly different. Complex spike responses, which proved to be sensitive to the amplitude, speed and direction of whisker movement, were evoked by only one or a few whiskers. Simple spike responses, which were not affected by the direction of movement, could be evoked by many individual whiskers. The receptive fields of Purkinje cells were largely intermingled, and we suggest that this facilitates the rapid integration of sensory inputs from different sources. Furthermore, we describe that individual Purkinje cells, at least under anaesthesia, may be bound in two functional ensembles based on the receptive fields and the synchrony of the complex spike and simple spike responses. The ‘complex spike ensembles’ were oriented in the sagittal plane, following the anatomical organization of the climbing fibres, while the ‘simple spike ensembles’ were oriented in the transversal plane, as are the beams of parallel fibres.
Encoding of whisker input by cerebellar Purkinje cells

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The cerebellar cortex is crucial for sensorimotor integration. Sensorimotor inputs converge on cerebellar Purkinje cells via two afferent pathways: the climbing fibre pathway triggering complex spikes, and the mossy fibre–parallel fibre pathway, modulating the simple spike activities of Purkinje cells. We used, for the first time, the mouse whisker system as a model system to study the encoding of somatosensory input by Purkinje cells. We show that most Purkinje cells in ipsilateral crus 1 and crus 2 of awake mice respond to whisker stimulation with complex spike and/or simple spike responses. Single-whisker stimulation in anaesthetised mice revealed that the receptive fields of complex spike and simple spike responses were strikingly different. Complex spike responses, which proved to be sensitive to the amplitude, speed and direction of whisker movement, were evoked by only one or a few whiskers. Simple spike responses, which were not affected by the direction of movement, could be evoked by many individual whiskers. The receptive fields of Purkinje cells were largely intermingled, and we suggest that this facilitates the rapid integration of sensory inputs from different sources. Furthermore, we describe that individual Purkinje cells, at least under anaesthesia, may be bound in two functional ensembles based on the receptive fields and the synchrony of the complex spike and simple spike responses. The ‘complex spike ensembles’ were oriented in the sagittal plane, following the anatomical organization of the climbing fibres, while the ‘simple spike ensembles’ were oriented in the transversal plane, as are the beams of parallel fibres.

Introduction

Integration of sensory and motor information is one of the most important tasks of the brain. The rodent whisker system combines relatively simple movements with direct sensory feedback (Vincent, 1913; Welker, 1964; Brecht et al. 2006; Kleinfeld et al. 2006), making it an ideal model system to study sensorimotor integration. The cerebellum, being centrally located in both sensory and motor pathways, is essential for sensorimotor integration (Ito, 2000; De Zeeuw & Yeo, 2005; Krakauer & Shadmehr, 2006). Both afferent pathways to the cerebellar cortex, the mossy fibre–parallel fibre and the climbing fibre pathway, convey sensory information from the whiskers (Kleinfeld et al. 1999), and cerebellar output can affect whisker movements (Esakov & Pronichev, 2001; Lang et al. 2006). The mossy fibre–parallel fibre pathway and the climbing fibre pathway converge on Purkinje cells, which are the sole output neurons of the cerebellar cortex.

Thus, Purkinje cells respond to mechanical stimulation of the whiskers (Axelrad & Crepel, 1977; Brown & Bower, 2001; Loewenstein et al. 2005; Holtzman et al. 2006; Ozden et al. 2009), but hardly anything is known about the functional properties of whisker encoding by Purkinje cells. We do not know, for instance, which parameters of whisker movement, like direction or speed, are encoded by Purkinje cells. Furthermore, while it has been shown that there are several areas of the cerebellar cortex that are devoted to the processing of sensory information from...
the whiskers (Shambes et al. 1978), it is unclear whether there is a somatotopic representation on the single-whisker level within these areas. Such an organization is prominent in other brain regions involved in whisker sensation, including the trigeminal nuclei, the thalamus and the somatosensory cortex (Woolsey & Van der Loos, 1970; Petersen, 2007).

Sensory input from the whiskers reaches the cerebellar cortex via several pathways. It enters the brainstem via the trigeminal nuclei (Torvik, 1956; Clarke & Bowsher, 1962). The inferior olive, where the climbing fibres originate, receives input directly from the trigeminal nuclei (Yatim et al. 1996). In addition, the output of the trigeminal nuclei enters the mossy fibre system via three pathways. There is a direct pathway (Yatim et al. 1996), a short pathway via the pontine nuclei (Swenson et al. 1984), and an indirect connection via the thalamo-cerebro-pontine loop (Kleinfeld et al. 1999) (Fig. 1A). While climbing fibre activity evokes complex spikes in the postsynaptic Purkinje cells, parallel fibre activity can modulate simple spike firing by Purkinje cells.

Here we studied Purkinje cell responses to mechanical whisker stimulation. We show that sensory input from the whiskers can evoke both complex spike and simple spike responses in Purkinje cells. Remarkably, in the anaesthetised mice the receptive fields of the complex spike and the simple spike responses of a Purkinje cell proved to be different. Simultaneous recordings of multiple Purkinje cells showed that functional ensembles of Purkinje cells cooperate in encoding sensory input. These cells were grouped in two different ways: in sagittal groups based on their complex spike pattern and in transverse groups based on their simple spike pattern.

Methods

Animals

Seven male C57BL/6 mice (6–31 weeks old; Harlan, Horst, The Netherlands) were anaesthetised with isoflurane (2% in O2) and received lidocaine (∼1 µg) subcutaneously at the surgical location. After reaching a surgical level of anaesthesia, a pedestal was attached to the skull with Optibond adhesive (Kerr Corporation, Orange, CA, USA). After recovery from anaesthesia, they were familiarised with the head-restraint and the recording setup during two or three sessions of 1 h each. On the day of the experiment, the mice were anaesthetised again with isoflurane and a craniotomy was made above crus 1 and crus 2 on the right side. Recordings started at least 30 min after recovery from anaesthesia.

For the electrophysiological experiments involving mechanical whisker stimulation, 41 male C57BL/6 mice (6–31 weeks old; Harlan) were initially anaesthetised with isoflurane (4% in O2) and subsequently deeply anaesthetised with a mixture of ketamine and xylazine (approx. 110 and 20 mg kg−1, respectively, administered intraperitoneally). At the surgical location, lidocaine (∼1 µg) was given subcutaneously. After reaching a surgical level of anaesthesia, the skin, skull and dura were removed above crus 1 and crus 2 of the right hemisphere. A small recording chamber was attached to the skull with dental cement and filled with 0.9% saline to avoid dehydration of the brain. Throughout the surgery and the subsequent experiment, the mice were kept under anaesthesia. Body temperature, heart rate (ECG) and respiratory frequency were monitored continuously (PowerLab 4/30, ADInstruments, Bella Vista, Australia). The body temperature was kept constant at 37°C and subsequent doses of anaesthesia were given intraperitoneally (approx. 60 mg kg−1 h−1 ketamine and 3 mg kg−1 h−1 xylazine). At the end of the experiments, the mice were killed by cervical dislocation under anaesthesia. All experimental procedures were in accordance with the ethical policy of The Journal of Physiology (Drummond, 2009). As required by Dutch legislation, the experiments were approved by the institutional animal welfare committee (Erasmus MC, Rotterdam, The Netherlands).

Neuronal tracing

Retrograde tracing was performed following injections in various regions of crus 1 and crus 2 of either a gold–lectin conjugate (n = 4) or the cholera toxin b subunit (CTb, n = 3) as described previously (Ruigrok & Apps, 2007). Briefly, mice were anaesthetised with ketamine–xylazine as described above. After making a craniotomy, the tracers were injected in the area where electrophysiological recordings were made, at a depth of approximately 500 µm. For gold–lectin, we used pressure injection of 50–100 nl suspension. CTb was applied by iontophoresis (10 min with 4 µA, anodal, pulsed current). After tracer injections, the mice were allowed to recover for 1 week. Subsequently, they were anaesthetised with pentobarbital (80 mg kg−1), administered intraperitoneally, and fixed by transcardial perfusion with 4% paraformaldehyde. The brains were removed, sliced (40 µm thick) and further processed histologically. From the labelling pattern in the inferior olive, coarse reconstructions of the sagittal zones in crus 1 and crus 2 were made (Apps & Hawkes, 2009).

Whisker stimulation

Whisker stimulation in awake animals was done by repeated air puffs. The air puffs (∼300 mbar (∼30 kPa), 5 ms) were given in a randomly timed manner using a MPFI-2 pressure injector (Applied Scientific Instrumentation, Eugene, OR, USA). The air puff was
delivered by a small tube (2 mm diameter), placed approximately 3 cm above the whiskers at a 50 deg angle (with respect to the body axis) to deflect the whiskers in the caudal direction. The air stream was directed away from the face, in order to avoid stimulation of the eye and/or other facial structures.

In anaesthetised mice, mechanical stimulations of the whiskers were applied using a combination of two programmable piezo linear drives (M-663, Physik Instrumente, Karlsruhe, Germany) placed perpendicular to each other, allowing accurate (0.1 μm precision) 2-D manipulation of the whiskers. The piezo drives were controlled using a custom-written LabView (National Instruments, Austin, TX, USA) algorithm.

For single-whisker stimulation, one whisker was placed in a fine capillary attached to the piezo drives. For multiple-whisker stimulation, the whiskers were placed in a small capillary attached to the piezo drives. Whisker stimulation occurred at 0.5 Hz (whisker mapping protocol, Fig. 7A–G) or 0.25 Hz (all other experiments). Unless stated otherwise, the extreme position (on average 7 deg deflection; range: 6–9 deg) was reached following a sine waveform in 62.5 ms, after which the whisker was kept at the extreme position for 250 ms and moved backed again in 62.5 ms. The piezo sliders were mounted on a 3-D translation stage (Luigs & Neumann, Ratingen, Germany), so that they could be positioned in such a way that the resting position(s) of the inserted whisker(s) were very close to the native position(s). Movements were either in a fixed direction (caudal) or in a random direction (chosen out of eight possible directions at 45 deg intervals).

Whisker position tracking

Whisker movement was monitored using a fluid-cooled, high-speed CCD camera (operating at 8 kHz full-frame (480 × 500 pixels) rate; A504k, Basler Vision Technologies, Ahrensburg, Germany). The whiskers were illuminated from below using a custom-designed LED panel. This LED panel worked at a wavelength (640 nm) that is hardly visible to mice (Jacobs et al., 2007). The positions of the whiskers were tracked using a custom-written LabView routine on a dedicated computer running under a real-time operating system (National Instruments).

Electrophysiology

Recording electrodes (2–5 MΩ) were made from quartz-coated platinum–tungsten fibre (outer diameter, 80 μm; Thomas Recording, Giessen, Germany) according to the design of Eckhorn (Mountcastle et al. 1991). The electrodes were placed in a 8 × 4 matrix (Thomas Recording), with an inter-electrode distance of 305 μm. Each electrode was placed individually in the vicinity of a Purkinje cell. The position of the electrode was reconstructed from photographs made of the site of entry and the actual depth (approx. 200–2000 μm) in the tissue. We measured the transversal distance between the site of entry of each electrode and the border between the hemispheres and the vermis as well as the sagittal distance between the site of entry and the border between crus 1 and crus 2 from the photographs (see Fig. 1B). These coordinates were stored and adjusted to a typical microscopical image of a mouse for visualization of the recording sites. Up to 24 electrodes were used simultaneously. The electrophysiological signal was digitised at 12–25 kHz, using a 20–6000 Hz band-pass filter, amplified and stored using a RZ2 multi-channel workstation (Tucker-Davis Technologies, Alachua, FL, USA). Typical recordings were stable for several tens of minutes, while occasionally stable recordings of more than 2 h could be obtained (see also Supplemental Fig. S1).

Electrophysiological data analysis

Neuronal recordings were analysed online using OpenEx software suite (Tucker-Davis Technologies), and later re-analysed offline using custom-written software in LabView. Putative events were first detected using a combination of an amplitude threshold and a time-variant filter (‘Gabor’ method). Next, all putative events were superimposed and a waveform analysis under full visual control was used to select true action potentials. For each Purkinje cell, we constructed a complex spike-triggered simple spike histogram to test for the presence of a pause in simple spike firing following each complex spike (Granit & Phillips, 1956; Bell & Grimm, 1969). Only recordings that had a complex spike pause of at least several milliseconds (complete pause >4 ms, see Fig. 1E) were considered to be ‘single units’ and only these recordings were used for further analysis. When, during a recording, the signal-to-noise ratio decreased, analysis was stopped when there was either no longer a complex spike pause, or when the signal-to-noise ratio no longer allowed reliable detection of spikes.

Statistical significance of peri-stimulus histograms and cross-correlograms was evaluated by defining a control period, during which we calculated the average and the 99% confidence interval. A response or correlation was considered ‘significant’ when it exceeded the threshold during at least one bin of the ‘responsive period’, as defined below. In order to control for ‘false positives’, we searched for threshold crossings during a time interval, equally long as the responsive period, during the control period. Under most conditions, the 99% confidence interval was not associated with any ‘false positives’, but for a subset of
experimental groups, the 99% confidence interval was not satisfactory and we adjusted the threshold as described below.

**Purkinje cell responses to whisker stimulation**

From each recording, two peri-stimulus histograms were made: one for the complex spikes and one for the simple spikes, both with 20 ms bin width. The onset of the whisker movement was at $t = 0$ ms. The 1 s pre-stimulus interval was considered as the control period, and a response was marked as significant if the peri-stimulus histogram exceeded the 99% confidence interval during at least one 20 ms bin during a defined post-stimulus interval: complex spike response, 0–140 ms after the onset of the stimulus; early-positive simple spike response (0–20 ms); early-negative simple spike response (0–60 ms); late-positive simple spike response (20–200 ms); and late-negative simple spike response (60–200 ms). No threshold crossings were detected during the pre-stimulus interval.

In order to test for direction selectivity, we tested more trials (up to around 1000) and first tested for each Purkinje cell whether the complex and/or simple spikes were significantly modulated by whisker stimulation. Next, we defined the responsive period per Purkinje cell as the time during which the response exceeded the threshold. In these initial steps, we used the aggregate threshold for all directions. Subsequently, we made peri-stimulus histograms for all eight directions and measured the response during the responsive period as a percentage of the firing rate during the 1 s pre-stimulus interval.

To test for direction selectivity, we used the technique of 'directional testing'. We applied the whiskers away: 0 positive, 1 tested (D1); 2 whirlers away: 0 positive, 2 tested (A1 and C1); 2 whiskers away in a 'narc': 1 whisker away: 0 positive, 2 tested (A1, B1, C1 and D1, and only B1 gave a significant response, this would lead to the following score (for the row): 3 whiskers away: 0 positive, 2 tested; 7.5% had a response between 2.8 and 3.2 S.D.; for late-positives this number was 8 out of 228 (3.5%). In order to exclude 'false negatives' for these categories we lowered the threshold to 2.8 S.D., but rejected experiments in which the threshold was crossed during the baseline recording.

We characterised the 'receptive field' per Purkinje cell and response category by starting at the first responsive period and checking whether a response was also recorded from the neighbouring whiskers, lying in the same row. Next, the whiskers, still in the same row, but now two places further on were tested, etc. The same procedure was used for the arcs (see Fig. 4A). In all cases, we divided the number of 'positive' whiskers for each location (e.g. '2 whiskers away in an arc') by the total number of whiskers tested for that condition. For instance, if we were able to test A1, B1, C1 and D1, and only B1 gave a significant response, this would lead to the following score (for the arc): 1 whisker away: 0 positive, 2 tested (A1 and C1), 2 whiskers away: 0 positive, 1 tested (D1), 3 whiskers away: 0 positive, 0 tested.

**Coherent complex spike firing and synchrony**

For pairs of Purkinje cells recorded simultaneously for at least 600 s, we constructed cross-correlograms of the complex spike times using a custom-written LabView routine. The threshold was set at the median number of complex spikes + 5 S.D. of the $-3$ to $-2$ s interval. A pair of Purkinje cells was considered to fire coherently if any of the 10 ms bins between $-100$ and $+100$ ms exceeded this threshold. If one, or both, of the bins next to 0 ms exceeded the threshold, we considered that Purkinje cell pair to fire synchronously. We discriminated between 10, 5 and 2 ms synchrony, according to the bin size at which synchrony could still be measured. At 1 ms bins, binning artefacts were so prominent that we refrained from a quantitative analysis of '1 ms synchrony'. The synchrony index was calculated as described in De Zeeuw et al. (1997).

**Synchrony and sensory input**

We selected all Purkinje cell pairs of which both Purkinje cells showed strong complex spike responses to stimulation of the same whisker (response $> 5$ S.D. of baseline). For these pairs, joint peri-stimulus histograms...
of the complex spike times were constructed with a custom-written LabView algorithm based on the method described by Aertsen et al. (1989). The histogram over the 45 deg line (containing the synchronous events) was normalized for the changes in complex spike frequency during whisker movement as described by Gerstein (1998).

Simple spike synchrony

Cross-correlograms were made of the simple spike times of pairs of Purkinje cells (>600 s of recording), using 99 bins of 10 ms. The firing pattern was considered to be synchronous if the centre bin exceeded the average baseline firing rate + 2 s.d., corresponding to the 95% confidence interval. As baseline, we considered the interval from −500 to −150 ms.

Statistics

Unless stated otherwise, the data are presented as mean value ± s.d. The statistical tests and threshold for significance used are mentioned in the text and/or figure legends where applicable.

Results

Multiple single-unit recordings of Purkinje cells in vivo

We studied Purkinje cell responses to sensory stimulation of the whiskers. To this end, we made extracellular multiple single-unit recordings of cerebellar Purkinje cells in 7 awake, head-restrained adult, male C57BL/6 mice and in 41 mice under ketamine–xylazine anaesthesia. Quartz-coated platinum–tungsten electrodes were placed in crus 1 and crus 2 of the cerebellar cortex ipsilateral to the stimulated whisker(s) (Fig. 1B). Purkinje cells produce two types of spikes: relatively rare complex spikes (awake mice: frequency \( f = 0.8 \pm 0.4 \, \text{Hz} \); with ketamine–xylazine anaesthesia: \( f = 0.6 \pm 0.5 \, \text{Hz} \)) and frequent simple spikes (\( f = 50 \pm 12 \, \text{Hz} \) and \( f = 40 \pm 21 \, \text{Hz} \), respectively) (Fig. 1C). Complex spikes are followed by a pause in simple spike firing of at least several milliseconds (Fig. 1D and E). The occurrence of such a ‘complex spike pause’ is the hallmark of a single-unit recording of a Purkinje cell (McDevitt et al. 1982; Simpson et al. 1996). Only single-unit recordings of Purkinje cells were used for this study. We were able to measure up to around 15 Purkinje cells simultaneously.

Purkinje cell responses to whisker stimulation in awake mice

Air puffs applied to the whisker pad elicited neuronal responses in 20 out of 24 (83%) Purkinje cells in ipsilateral crus 1 and crus 2 of awake mice (Fig. 2). Of these 20 responsive Purkinje cells, 14 (70%) showed both a complex spike and a simple spike response to whisker stimulation, 2 (10%) only a complex spike response and 4 (20%) only a simple spike response.

In order to increase the visibility of the whisker movements, we trimmed the out-of-focus whiskers prior to the experiment. The positions of the remaining whiskers (on average \( 6 \pm 2 \, \text{mean} \pm \text{s.d.} \), Fig. 2A) were tracked automatically (Fig. 2B top). The air puffs were directed at the whisker pad, away from the face to avoid stimulation of the eye and/or other facial structures. In most Purkinje cells (13/16 (81%)), the complex spike response had a latency of maximally 40 ms. The other three cells had a latency of 100 ms. Of the 13 'short-latency' cells, 5 showed an additional second peak. Taken together, the average peri-stimulus histogram shows a clear bi-modal distribution (Fig. 2G).

Simple spike responses were even more variable between individual Purkinje cells than were complex spike responses. Some cells (4/18 (22%)) showed a decrease in simple spike firing, others (11 (61%)) an increase and the rest (3 Purkinje cells (17%)) a combination of both. Particularly striking was the response pattern of the Purkinje cell illustrated in Fig. 2D and F, which showed a tri-phasic response. An initial increase in simple spike firing was directly followed by a period of decreased simple spike firing, after which there was a second, prolonged period of increased simple spike firing. The kinetic properties of the Purkinje cell responses have been summarized in Table 1.

Whisker-induced decrease in simple spike firing can be independent of complex spikes

In response to air puff stimulation of the whiskers in awake mice, we found in eight Purkinje cells both a complex spike response and a decrease in simple spike firing (Figs 2C–H and 3A). We did not find a Purkinje cell showing a dip in the simple spike rate without also showing a complex spike response. Since complex spikes cause a temporary cessation of simple spikes (Fig. 1C–E), we wondered whether the sensory-induced decreased simple spike firing could be attributed to complex spike firing. To answer this question, for each Purkinje cell recording we separated the trials during which there was a complex spike during the responsive period. For this analysis, the responsive period was defined as the period during which the complex spike response exceeded the threshold (see
Fig. 2E). Next, we constructed raster plots of the simple spike times of all trials with a complex spike (Fig. 3B) and of all trials without a complex spike (Fig. 3C). As can also be seen in the associated peri-stimulus histograms (Fig. 3D), the Purkinje cell in the left column shows a whisker stimulation-induced decrease in simple spike firing also in the absence of a complex spike. In contrast, the decrease in simple spike firing observed in the Purkinje
cell in the right column was present only in the trials with a complex spike. Of the eight Purkinje cells that had a whisker stimulation-induced decrease in simple spike firing, six cells showed whisker stimulation-induced simple spike inhibition uncorrelated to the occurrence of complex spikes. In the other two recordings, the simple inhibition was tightly correlated to the timing of the complex spikes. Thus, we conclude that, while the complex spike pause can contribute to sensory-induced simple spike inhibition, it cannot explain the majority of the inhibition. Possibly, the inhibitory interneurons of the molecular layer are important for this simple spike inhibition.

In conclusion, most Purkinje cells in crus 1 and crus 2 responded to air puff stimulation of the whiskers. However, there was a large variation in neuronal responses, possibly due to the relatively poor reproducibility of air puff stimulation. Therefore, we decided to continue studying Purkinje cell responses to whisker input using accurate piezo-actuators to stimulate single whiskers in mice under ketamine-xylazine anaesthesia.

Complex spike responses to whisker stimulation in anaesthetised mice

We made single-unit recordings of 74 Purkinje cells in ipsilateral crus 1 and crus 2 during single-whisker stimulation (of whisker C2; in random directions). In seven Purkinje cells (9%) a significant complex spike response was recorded (Fig. 4, Table 1). On average, the complex spike response started 59 ± 27 ms after the onset of the movement. In responsive Purkinje cells, the chance of complex spike firing upon whisker stimulation was 4.6 ± 2.1 times the baseline frequency. Even in responsive cells, complex spike firing occurred in a subset of trials (9 ± 10%). Typically, a response to whisker stimulation consisted of only one complex spike. Sometimes a second, smaller peak was observed in the peri-stimulus histogram, around 100 ms after the first peak. Since this time interval is much larger than the duration of the movement (62.5 ms), it is unlikely that it reflects an off-response. A similar, rhythmic response has, however, been reported previously for forepaw stimulation in cats (Bloedel & Ebner, 1984) and eye-blink stimulation in mice (Van Der Giesen et al. 2008).

Strikingly, the backward movement generally did not evoke a complex spike response (Fig. 4F and I). Since we used eight different directions of whisker movement, alternated in a random fashion, the lack of a complex spike response to the backward movement cannot be explained by a preference for a certain direction of movement (see Fig. 5). Thus, we conclude that repeated stimulations, shortly after each other, fail to elicit complex spike responses.

For comparison, we also recorded 68 Purkinje cells during which multiple whiskers were stimulated simultaneously: γ, C1 and C2 (and sometimes also C3). Remarkably, the percentage of responsive Purkinje cells was between three and four times as high as for single-whisker stimulation (22 out of 68 (32%) vs. 7 out of 74 (9%) Purkinje cells, P = 0.0008, Fisher’s exact test). Neither the latency (45 ± 16 vs. 59 ± 27 ms, P = 0.1129, Student’s t-test) nor the percentage of successful trials (13 ± 11 vs. 9 ± 10%, P = 0.3474, Student’s t-test) differed significantly between multiple- and single-whisker stimulation. This suggests that stimulating more whiskers increases the number of responsive Purkinje cells, but not the amplitude nor the kinetics of the complex spike response in a given Purkinje cell.

Simple spike response to whisker stimulation in anaesthetised mice

Next, we characterised the simple spike response to single-whisker stimulation. We found that, just as air puffs in awake mice (Fig. 2), single-whisker stimulation in anaesthetised mice could induce both increased and decreased simple spike firing. Of both types of modulation, there were an early and a late phase (Fig. 4E, G and I, Table 1). Overall, the sequence was as follows: early-positive, early-negative, late-positive and late-negative response. Although most Purkinje cells showed a combination of some of these four response types, none showed all four. The fraction of Purkinje cells with simple spike responses to single-whisker stimulation was, as it was for complex spike responses, rather low (Table 1). The chance of finding a simple spike response in a given Purkinje cell did not depend on whether or not there was a complex spike response in that Purkinje cell.

The early-positive simple spike response typically consisted of a short response with a short latency, always occurring during the first 20 ms bin of the peri-stimulus histogram. The variation in latency and duration of the later phases of simple spike responses was considerably larger. Remarkably, the early responses were more prominent upon single-whisker stimulation, while the late responses were mainly observed during multiple-whisker stimulation (Table 1).

Direction selectivity

Next, we further characterized which parameters of whisker movement were relevant for the Purkinje cell response. The direction of whisker movement conveys important information about the environment. Hence, in several brain regions, neuronal responses relate to the direction of whisker movement (Bale & Petersen, 2009).
Early-positive simple spike response (0–20 ms)

Late-positive simple spike response (20–200 ms)

Late-negative simple spike response (60–200 ms)

parameters were not significant (Student's t test). For comparison, the response parameters to air puff stimulation in awake mice are also shown. Because of the qualitative differences in stimulation protocols, we did not test for significant differences between responses in awake and anaesthetised mice. Values are the mean ± S.D.

Table 1. Response kinetics

<table>
<thead>
<tr>
<th>Category</th>
<th>Anaesthesia</th>
<th>PCs tested</th>
<th>Responsive PCs</th>
<th>Latency (ms)</th>
<th>Duration of response (ms)</th>
<th>Response amplitude (% of baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex spike response (0–140 ms)</td>
<td>C2 yes 74</td>
<td>7 (9.5%)*</td>
<td>59 ± 27</td>
<td>30 ± 17</td>
<td>459 ± 211</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-row yes 68</td>
<td>22 (32.4%)</td>
<td>45 ± 16</td>
<td>40 ± 15</td>
<td>702 ± 449</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air puff no 24</td>
<td>16 (66.7%)</td>
<td>51 ± 38</td>
<td>27 ± 13</td>
<td>994 ± 1093</td>
<td></td>
</tr>
<tr>
<td>Early-positive simple spike response (0–20 ms)</td>
<td>C2 yes 74</td>
<td>6 (8.1%)*</td>
<td>0 ± 0</td>
<td>20 ± 0</td>
<td>122 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-row yes 68</td>
<td>0 (0.0%)</td>
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<td>—</td>
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<td></td>
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<td></td>
<td>Air puff no 24</td>
<td>1 (4.2%)</td>
<td>0</td>
<td>20</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Early-negative simple spike response (0–60 ms)</td>
<td>C2 yes 74</td>
<td>12 (16.2%)</td>
<td>27 ± 10</td>
<td>25 ± 9</td>
<td>78 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-row yes 68</td>
<td>5 (7.4%)</td>
<td>36 ± 9</td>
<td>28 ± 11</td>
<td>85 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air puff no 24</td>
<td>6 (25.0%)</td>
<td>13 ± 10</td>
<td>33 ± 16</td>
<td>58 ± 27</td>
<td></td>
</tr>
<tr>
<td>Late-positive simple spike response (20–200 ms)</td>
<td>C2 yes 74</td>
<td>2 (2.7%)*</td>
<td>100 ± 28</td>
<td>20 ± 0</td>
<td>112 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-row yes 68</td>
<td>9 (13.2%)</td>
<td>78 ± 21</td>
<td>40 ± 26</td>
<td>117 ± 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air puff no 24</td>
<td>14 (58.3%)</td>
<td>56 ± 20</td>
<td>127 ± 243</td>
<td>148 ± 35</td>
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</tr>
<tr>
<td>Late-negative simple spike response (60–200 ms)</td>
<td>C2 yes 74</td>
<td>0 (0.0%)*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-row yes 68</td>
<td>5 (7.4%)</td>
<td>100 ± 28</td>
<td>48 ± 23</td>
<td>80 ± 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air puff no 24</td>
<td>5 (20.8%)</td>
<td>96 ± 36</td>
<td>64 ± 36</td>
<td>62 ± 28</td>
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</tbody>
</table>

Purkinje cell responses to single- and multiple-whisker stimulation (of C2 and C-row (consisting of γ, C1, C2 and sometimes C3), respectively) in mice under anaesthesia. The stimulation protocol was the same as shown in Fig. 4: 350 trials at 0.25 Hz in random directions. Responsive periods were considered to be the interval during which the spike rate passed the threshold (see Methods). Statistical comparisons between single- and multiple-whisker stimulations were made: *P < 0.05, Fisher's exact test; the other parameters were not significant (Student's t test). For comparison, the response parameters to air puff stimulation in awake mice are also shown. Because of the qualitative differences in stimulation protocols, we did not test for significant differences between responses in awake and anaesthetised mice. Values are the mean ± S.D.

We tested whether complex spikes and simple spikes also conveyed information about the direction of whisker movement. To this end we deflected one (C2) or multiple (C-row) whiskers in eight directions (see Fig. 4A).

We recorded the complex spike response to whisker stimulation, and constructed polar plots showing the complex spike response for each of the eight directions used (Fig. 5A). In total, we tested 15 Purkinje cells with a significant complex spike response. The polar plots of these cells had on average an octagonality value of 0.79 ± 0.08, which was significantly smaller than that of a regular octagon (P < 0.001, one-sample t test). This implies that the occurrence of a complex spike response to mechanical whisker stimulation depended on the direction of movement. Of the 15 Purkinje cells tested, 6 'favoured' the dorso-caudal direction (Fig. 5B). In contrast, the simple spike responses did not show any directional selectivity (Fig. 5C).

**Figure 2. Whisker stimulation triggers Purkinje cell responses in awake mice**

A, photograph of the whiskers of a mouse. The whiskers located outside the focal plane were cut. The movement of the whiskers along the green line was tracked at 1 kHz. Whisker position was defined as the intersection point of the green line and the whisker. In this experiment, 6 whiskers could be tracked. B, top: positions of the 6 whiskers indicated in A. The colours of the traces correspond to those of the circles in A. The 'yellow' whisker moved out of the field of view during air puffs. The timing of the air puffs is indicated by the two black lines. Bottom: the corresponding electrophysiological recording of a Purkinje cell. Complex spikes are indicated by an asterisk. Raster plots of the time stamps of complex spikes (C) and simple spikes (D) during 556 trials. Time t = 0 s indicates the onset of the air puffs. Peri-stimulus histograms of the complex spikes (E) and simple spikes (F). In this Purkinje cell, the complex spike response was mono-phasic, while the simple spike response was tri-phasic: an early-positive response (1) was followed by an early-negative response (2). Finally, there was a late-positive response (3). The data in panels A–F originate from the same experiment. G, average peri-stimulus of histogram of all 16 Purkinje cells showing a complex spike response to air puff whisker stimulation. Inset of the 24 Purkinje cells recorded in crus 1 and crus 2, 16 showed a complex spike response to whisker stimulation. H, average peri-stimulus of histogram of all 18 Purkinje cells showing a simple spike response to air puff whisker stimulation. Inset: of the 24 Purkinje cells recorded in crus 1 and crus 2, 18 showed a simple spike response to whisker stimulation.
Figure 3. Sensory induced simple spike inhibition can be independent of climbing fibre activity in awake mice.

A, peri-stimulus histograms of two representative Purkinje cells. Both Purkinje cells responded to repeated air puff stimulation (at $t = 0.0$ ms) with complex spike (blue) and simple spike (red) responses. The left Purkinje cell received 338 stimuli, the right Purkinje cell 490. They were recorded from the same mouse and for a large part simultaneously. B, raster plots of simple spike times of all trials with a complex spike response, i.e. with a complex spike during the responsive period (cf. A). C, raster plot of an equal amount of trials as in B, but now from trials without a complex spike response. It can be seen that the simple spike inhibition seen in B is still present in the left Purkinje cell, but not in the right Purkinje cell. Thus, the complex spike pause cannot be the sole cause of sensory-induced simple spike inhibition in the left Purkinje cell. D, peri-stimulus histograms of the simple spike times, comparing the trials with (filled boxes) and without (open boxes) a complex spike response. In 6 out of 8 Purkinje cells, the whisker stimulation-induced simple spike inhibition was not correlated to the complex spike response, when studied at a trial-by-trial base (left column), as it was in the other 2 Purkinje cells (right column).
Activation threshold for complex spike responses

Next, we wondered to which extent amplitude and speed of the whisker movement contributed to the complex spike response. We employed an experimental protocol in which we systematically varied the amplitude of the whisker movement and the time interval needed to reach the maximal amplitude. We used 16 different stimuli, with four values for the amplitude and four for the time interval. A representative example is shown in Fig. 6A. Statistical analysis (Fig. 6B and C) was performed on the data from seven Purkinje cells.

An increase in the time to reach the maximal angular velocity (ωmax) postponed the onset of the complex spike response (Fig. 6B). For relatively short times to ωmax, the latency of the complex spike response was changed proportionally to the time to ωmax, irrespective of the absolute amplitude of the response. During very slow movements, the latency of the complex spike response was no longer fully linear to that of the time to ωmax: the complex spike response often occurred before the ωmax was reached (Fig. 6B). The (angular) velocity depends both on the distance (angle) travelled and the time required to do so. A doubling of the amplitude, within the same time interval, increased the number of complex spikes during the response by 87 ± 25% (mean ± S.E.M., Fig. 6C). Halving the time interval, but maintaining the same amplitude, led to an increase of the number of complex spikes during the response by 41 ± 16% (mean ± S.E.M., Fig. 6C). Thus, the number of complex spikes increased with larger angular velocity, whether this increase in velocity was achieved by a larger amplitude or by a shorter time of movement. However, the number of complex spikes was more strongly modified by changes in the amplitude than by changes in the interval time. In conclusion, a complex spike response was produced when the whisker movement exceeded a (velocity) threshold. Once the threshold had been exceeded, the chance of evoking a complex spike depended on both the velocity and the amplitude of the whisker movement. Of these latter two parameters, the amplitude had the strongest impact.

Receptive fields of Purkinje cells

To study the receptive fields of the Purkinje cells, we made recordings of Purkinje cells and stimulated individual whiskers consecutively. When we found a complex spike response to a certain whisker, we tested whether other whiskers also evoked a complex spike response in that same Purkinje cell. In most Purkinje cells, we could find only a single whisker that was able to generate a complex spike response, in line with Axelrad & Crepel (1977). If there was a second ‘active’ whisker, it was always located in the same row as the first whisker (Fig. 7B). The finding that most Purkinje cells reacted only to a single whisker is in line with our finding that multiple-whisker stimulation increased the number of responsive Purkinje cells, but not the amplitude of the response, when compared to single-whisker stimulation (Table 1). We conclude that with respect to complex spike responses, whiskers are largely independent of each other.

For simple spikes, the situation is clearly different. Most Purkinje cells, if they showed a simple spike response to one whisker, also showed simple spike responses to another whisker (Fig. 7C). Remarkably, we could not find a patch of adjacent ‘active’ whiskers. On the contrary, the chance of getting a simple spike response from the direct neighbour of an ‘active’ whisker was almost identical to that of getting a simple spike response from a whisker at the other end of the whisker pad. Furthermore, the whiskers in-between might perhaps not evoke a simple spike response at all. Thus, while Purkinje cells had a small and clearly defined complex spike receptive field, simple spike receptive fields were larger and did not seem to have a strict structural organization.

In the abovementioned simple spike analysis, we did not take the different types of simple spike modulation (Table 1) into account. Therefore, we repeated the receptive field analysis for each of the four types of simple spike modulation. Late-negative responses were very rare (2 out of 228 whiskers tested (0.9%)), so they were not further analysed. For each of the three other response types, early-positive, early-negative and late-positive responses, we found qualitatively the same results as with the initial simple spike analysis: each Purkinje cell has a large simple spike receptive field without a clear ordering pattern.

The receptive fields of the three types of simple spike modulation were highly correlated, but not identical. This implies that different types of simple spike modulation to stimulation of a certain whisker were often, but not always, manifest together in a single Purkinje cell. For instance, of all 228 whiskers tested, 42 (18%) evoked an early-positive simple spike response and 65 evoked an early-negative response (29%); 26 whiskers evoked both responses, a fraction much larger than expected by chance ($P = 0.0007$, Fisher’s exact test). In contrast, the incidence of complex spike responses was not related to the simple spike response ($P = 0.1430$ when compared to the incidence of early-positive responses, $P = 0.6074$ (early-negative responses) and $P = 0.2303$ (late-positive responses), Fisher’s exact test). Thus, complex spike and simple spike receptive fields were markedly different and not related to each other. Subsequently, we calculated the fraction of Purkinje cells in which we could elicit a response by stimulating the individual whiskers (Fig. 7D and E).

Finally, we projected the principal whisker for each recorded Purkinje cell to the surface of crus 1 and crus 2.
Figure 4. Single-whisker stimulation affects both complex spike and simple spike firing in anaesthetised mice

A, schematic drawing of the organization of the mouse mystacial pad, showing the relative positions of the whiskers used in this study. B, upper trace: programmed trajectory of the C2 whisker, which was attached to a piezo drive. Lower trace: extracellular recording of a Purkinje cell, showing both complex and simple spikes. Note that complex spikes (∗) occur shortly after the start of the whisker movement. C, whisker movement tracked with a high-speed CCD camera (sample frequency, 1.0 kHz). Time scale as in D–I. D, raster plot showing the timing of complex spike firing. During each trial, we stimulated whisker C2 according to trajectory depicted in C, but in a random direction. E, as in D but for the simple spikes. Note that the average firing frequency showed some long-term changes, possibly related to variations in the state of anaesthesia (see also Supplemental Fig. 1). The simple spike response was, however, present in periods with higher as well as with lower basal firing rate (data not shown). F, peri-stimulus histogram of the complex spike firing in a representative experiment following stimulation of whisker C2 (777 trials). The largest response was during the movement from the resting position to the extreme position (1). In some experiments, including this one, a second peak was observed around 100 ms.
The principal whisker was defined as the whisker eliciting the largest response. For the simple spike response, we took the early-negative response as a measure for the principal whisker, since this was by far the most abundant type of simple spike response. For both complex spike and simple spike responses there was a highly fragmented map without any clear pattern (Fig. 7F and G). Hence, we conclude that the organization of the whisker-sensitive areas in crus 1 and crus 2 does not follow a somatotopic organization, as for instance found in layer IV of the barrel cortex (see Petersen, 2007).

**Purkinje cell locations in relation to sagittal zones**

In order to further characterize the locations of Purkinje cells receiving sensory input from the whisker system, we made recordings of 134 Purkinje cells during single-whisker (C2) or multiple-whisker stimulation (C-row). For each Purkinje cell, we made 350 whisker movements (at 0.25 Hz) in randomised directions. In a surface projection of the locations of these Purkinje cells in crus 1, it can be seen that Purkinje cells showing complex spike modulation to whisker stimulation are predominantly found in the central area of crus 1 (Fig. 7H, Supplemental Fig. S2). In the medial part of crus 1, i.e. the part bordering the vermis, only a few Purkinje cells showed complex spike modulation. Purkinje cells showing simple spike modulation were distributed more evenly along the medio-lateral axis of crus 1 (Fig. 7I, Supplemental Fig. S2). The distribution of responsive Purkinje cells in crus 2 lacked obvious clustering, both for the complex spike and the simple spike responses (Fig. 7H and I).

Next, we wanted to know how the locations of Purkinje cells responding to whisker stimulation relate to the organization of the cerebellar cortex. It is well known that the organization of cerebellar climbing fibres follows a sagittal zonal pattern, which has been most thoroughly described for the rat (e.g. see Sugihara et al. 2004; Voogd & Ruigrok, 2004; Pipers et al. 2005), but can also be distinguished in several other species including the mouse (Groenewegen et al. 1979; Garwicz, 1997; Edge et al. 2003; Schoneville et al. 2006b; Sugihara & Quy, 2007). Based on these studies, a general scheme of the sagittal zones was recently provided by Apps & Hawkes (2009). The position of the recordings sites was verified by analysing the labelling patterns from a series of seven micro-injections with retrograde tracers at different locations in crus 1 and crus 2 (Fig. 8A). The labelling pattern obtained in the contralateral inferior olive (Fig. 8C and D) as well as the general scheme of olivo-cerebellar projections served as a tool to define the zonal boundaries within the crura (Fig. 8B). The patterns of retrograde olivary labelling showed that the central regions of the crura receive their climbing fibre afferents mostly from the principal olive (D-zones), whereas the more medially located Purkinje cells received their climbing fibres from the rostro-medial or rostral half of the dorsal and medial accessory olives, respectively (C-zones).

Retrograde labelling was also prominent in the ipsilateral trigeminal nuclei, mainly the principal nucleus (Pr5) and the spinal nucleus pars interpolaris (Sp5i) (Fig. 8E–H). In line with the results of Yatim et al. (1996), we found that the dorsal parts of Pr5 and Sp5i projected mainly to the central part of crus 1 and crus 2, while the ventral parts projected more to the medial part (bordering the vermis) and lateral parts of crus 1 and crus 2. In addition, heavy labelling was also noted within the contralateral pontine nuclei (Fig. 8I and J), indicating that the pontine nuclei also project to crus 1 and crus 2. The contralateral trigeminal nuclei and the ipsilateral pontine nuclei also contained retrogradely labelled neurons, but to a much less extent (data not shown).

Complex spike responses were particularly often recorded from the central part of crus 1, and less often from the part bordering the vermis (Fig. 7H, Supplemental Fig. S2). This distribution mirrors the trigemino-olivary projections: the spinal nucleus projects mainly to the rostro-medial part of the dorsal accessory olive and the dorso-medial group and ventral leaf of the principal olive (Uzeman, 1960; Huerta et al. 1983; De Zeeuw et al. 1996; Yatim et al. 1996). These regions project to the C3, D1 and D0 zones (Voogd & Ruigrok, 2004; Sugihara & Quy, 2007). As some complex spike responses to mechanical whisker stimulation were also found in the medial part of crus 1, a contribution from the so-called A2 zone, supplied by medial aspect of the caudal part of the medial accessory olive, cannot be excluded (see Figs 7H and 8B).
Figure 5. Direction selectivity of Purkinje cell responses in anaesthetised mice

A, complex spike responses depended on the direction of whisker movement. Eight peri-stimulus histograms show the complex spike responses for each direction of a typical Purkinje cell. For this Purkinje cell, stimulation of whisker C2 in the dorsal direction had the largest impact. The polar plot shows the number of complex spikes during the response period (the light blue bars in the peri-stimulus histograms) per direction (light blue line), as well as the complex spikes during the same time interval before the onset of the whisker movement (dark blue line). B, the dorso-caudal direction was most often found to be the ‘favourite’ direction of the Purkinje cells. The arrow indicates the number of Purkinje cells for which each direction evoked the largest complex spike response. C, while complex spike responses depended on the direction of the movement, simple spike responses did not. This proved to be true for all four kinds of simple spike responses: early-positive, early-negative, late-positive and late-negative responses. An ‘octogonality’ value (see Methods section) of 1.0 implies no direction selectivity at all. For this analysis, we measured complex spike responses from 15 Purkinje cells, and simple spike responses from 7, 9, 7 and 5 Purkinje cells for the early-positive, early-negative, late-positive and late-negative responses, respectively.
(Voogd & Ruigrok, 2004). Simple spike responses to whisker stimulation were found in Purkinje cells dispersed over crus 1 and crus 2 (Fig. 7I, Supplemental Fig. S2), corresponding to the pattern of the trigemino-cerebellar mossy fibres, that originate from both the spinal and the spinal trigeminal nuclei and innervate virtually all of crus 1 and crus 2 (Yatim et al. 1996).

**Coherent firing of Purkinje cell pairs**

Purkinje cells can cooperate in anatomically and functionally defined ensembles. We investigated coherent complex spike firing in our recordings to further define the extent to which Purkinje cells are functionally related to each other in crus 1 and crus 2 (Fig. 9A and B). We made cross-correlograms of the complex spike times of each Purkinje cell pair for which we had at least 600 s of simultaneous recording (Fig. 9C and D). Of the 295 Purkinje cell pairs tested, 124 (42%) showed a non-uniform cross-correlogram, indicating that the complex spike firing of these Purkinje cell pairs was not random with respect to each other. Correlated firing does not necessarily imply synchronous firing. Therefore, we made cross-correlograms with different bin sizes and tested whether one or both of the two bins around 0 ms exceeded the threshold, which was defined as the average + 5 S.D. of the interval −3 to −2 s. This led to three categories of synchrony: where complex spikes had an increased

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**Figure 6. Complex spikes encode the amplitude and speed of whisker movement in anaesthetised mice**

A, complex spike peri-stimulus histograms of a representative Purkinje cell recording during which the amplitude and time to maximal deflection of the whisker movement were systematically varied. Going from left to right, each next column represents data obtained with a doubled stimulus amplitude. Going from top to bottom, each next row represents data obtained with stimuli using half the time to reach the maximal deflection. Consequently, the diagonals (from bottom-left to top-right) have the same angular speed (ω). The green lines show the programmed whisker trajectories. In each histogram, the red line depicts the threshold (upper border of the 99% confidence interval). In the histograms marked with a coloured background, the response threshold was exceeded. From this experiment, it can be concluded that complex spike firing depends on a velocity threshold rather than an amplitude threshold.

B, for shorter time intervals, the increase in time to maximal angular velocity (ωmax) is linear with the increase in latency time. For longer time intervals, the latency does not increase linearly anymore. This indicates that for relatively fast movements, the complex spikes encode the maximal angular velocity, but for very long-lasting movements, complex spikes also occur before the maximal angular velocity is reached. C, doubling both the amplitude of a movement and the time to complete that movement does not result in a change in ωmax. Accordingly, the number of complex spikes evoked was unchanged (left bar). Doubling the amplitude, but keeping the time interval constant, was more effective in evoking complex spikes (middle bar) than halving the time, but maintaining the same amplitude (right bar). The analysis shown in B and C is based on the data from 7 Purkinje cells.
Chapter 3

A

B

C

D

E

F

G

H

I

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chance to occur within 10, 5 or 2 ms of each other. In total, 72 (24%) Purkinje cell pairs showed ‘10 ms synchrony’, of which 46 (16% of all pairs) showed ‘5 ms synchrony’ and 27 (9% of all pairs) ‘2 ms synchrony’ (Fig. 9F).

Thus, although we did find well-timed (2 ms) complex spike synchrony in 9% of the Purkinje cell pairs, in 33% of the Purkinje cell pairs there was coherent complex spike firing, but this was not truly synchronous. We wondered whether we could find any evidence for a travelling wave of complex spike activity over the cerebellar cortex. To test this, we plotted the distance between the cell pairs against the time of the maximum correlation (10 ms bins). The longer the distance between two Purkinje cells, the longer the average time between complex spikes (P = 0.0004, linear regression, slope = 14.7 ms mm⁻¹, Fig. 9G). Next, we investigated whether we could find any difference between the distance in transversal (medio-lateral) or sagittal (rostro-caudal) direction or in depth. We found no correlation between either transversal or sagittal distance and latency time (P = 0.1143 and P = 0.0854, respectively, linear regression, data not shown). However, when we looked at differences in the depth, we found that Purkinje cells that were situated at a deeper location tended to fire prior to more superficially located Purkinje cells (P = 0.0040, slope = −12.3 ms mm⁻¹, linear regression, Fig. 9H). Hence, we conclude that the difference in depth is the most important factor in determining the relative timing of Purkinje cells.

Simple spike synchrony, while prominent in the paramedian lobule, has been shown to be virtually absent in crus 2 (Heck et al. 2007). In line with this, we found little evidence for synchronous firing of simple spikes, except in Purkinje cell pairs that responded to the same whisker input. Of the 20 Purkinje cell pairs showing simple spike co-modulation, i.e. both Purkinje cells had simple spike responses to stimulation of the same whisker(s), 9 (45%) showed simple spike synchrony. Of the 45 Purkinje cell pairs tested that did not show this simple spike co-modulation, only 5 pairs (11%) showed simple spike synchrony (P = 0.0066, Fisher’s exact test, Fig. 9I).

In line with Heck et al. (2007), the extent of simple spike synchrony in crus 1 and crus 2 was relatively small, even in synchronously firing Purkinje cell pairs (Fig. 9I). The relative power of the synchrony was similar during periods of whisker movement and rest (Fig. 9J).

Complex spike synchrony and sensory input

Next, we investigated the impact of sensory input on coherent complex spike firing. To this end, we selected all simultaneously recorded Purkinje cell pairs which showed a complex spike response to whisker stimulation (15 out of 295 pairs (5%)). Indeed, 12 of these 15 ‘co-modulating’ Purkinje cell pairs (80%) showed correlated complex spike firing, compared to only 112 out of 280 other Purkinje cell pairs (40%) (P = 0.0028, Fisher’s exact test). In line with this, complex spike synchrony also occurred much more often in co-modulating than in other Purkinje cell pairs (9 out of 15 (60%) vs. 63 out of 280 (23%) pairs, respectively, P = 0.0027, Fisher’s exact test, 10 ms synchrony, data not shown).

Interestingly, when discriminating between Purkinje cell pairs of which both cells showed a strong complex spike response to whisker stimulation (> 5 S.D.) and the other co-modulating pairs (8 and 7 pairs, respectively), it became immediately apparent that the strongly co-modulating Purkinje cell pairs had a much higher incidence of synchronous firing than the weaker co-modulating Purkinje cell pairs (Fig. 10D). Indeed, complex spike synchrony in weakly co-modulating Purkinje cell pairs did not differ from that in non-co-modulating Purkinje cell pairs.

Figure 7. Receptive fields of Purkinje cells in anaesthetised mice

A, the follicles of the large vibrissae are ordered in a grid on the mystacial pad. For the experiments presented in this figure, we confined ourselves to the 14 whiskers depicted here. ‘Rows’ are lines of whiskers ordered in the rostro-caudal plane, ‘arcs’ are lines of whiskers ordered in the dorso-ventral plane. B, during a recording of a Purkinje cell, we tested one by one which of the whiskers elicited a complex spike response. For each responsive whisker, we tested whether the neighbouring whiskers also elicited complex spike responses. We discriminated between direct neighbours and neighbours two or three whiskers away, as well as between neighbours in the same row and in the same arc. C, as for B but for the simple spike responses. D, fraction of Purkinje cells in which a given whisker could elicit a complex spike response. For whiskers in arc ‘3’ we did not have enough data. E, as for D but for the simple spike responses. F, for each of the 28 Purkinje cells tested, the whisker that elicited the largest complex spike response is depicted as a coloured circle on the approximate location, as projected to the brain surface, in crus 1 or crus 2. Colour coding is the same as in A and D. (Near) overlapping locations have been displaced minimally to increase visibility. Purkinje cells that did not have a complex spike response to any of the whiskers tested are not shown. G, as for F but for the simple spike responses. Here, we illustrated the early-negative responses. H, surface-projected locations of Purkinje cells showing a complex spike response to whisker stimulation (filled symbols). Purkinje cells that did not show a complex spike response are indicated by open symbols. Circles: single-whisker stimulation (C2); bars: multiple-whisker stimulation (C-row). Most complex spike responses were found centrally in crus 1 (see also Supplemental Fig. 2). I, as for H but for the simple spike responses. The sagittal and transversal axes are indicated by R (rostral) and C (caudal), and by M (medial) and L (lateral), respectively.
Figure 8. Anatomical connections to crus 1 and crus 2

A, in 5 animals, we made in total 7 injections with retrograde tracers (4 gold–lectin and 3 cholera toxin b subunit (CTb)). The injection areas (which were ∼500 μm deep) are shown on a cross-section of the cerebellum. B, enlarged image of the right hemisphere. For each injection spot, we established in which sagittal zone(s) it was located on the basis of staining in the inferior olive. The grey lines indicate the most likely locations of the sagittal zones in crus 1 and crus 2. C and D, two representative images of the staining pattern in the inferior olive. The white lines delineate the contralateral inferior olive, the coloured lines indicate the stained area. Note that the colour coding...
pairs. At the same time, strongly co-modulating Purkinje cell pairs showed a strong increase in synchrony (7 out of 8 pairs (88%)) for 10 ms synchrony, compared to 63 out of 280 non-co-modulating cell pairs (23%), \( P = 0.0002; \) 5 out of 8 pairs (63%) for 5 ms synchrony, compared to 40/280 non-co-modulating pairs (17%), \( P = 0.0029; \) and 4 out of 8 pairs (50%) for 2 ms synchrony, compared to 23/280 non-co-modulating pairs (10%), \( P = 0.0033, \) Fisher’s exact test). Thus, we conclude that strongly co-modulating Purkinje cell pairs belong to functional ensembles of synchronously active Purkinje cells.

When two Purkinje cells showed a complex spike response to stimulation of the same whisker(s), they were most often found within a sagittal zone (Fig. 10E). Remarkably, when two Purkinje cells both showed a simple spike response to stimulation of the same whisker(s), they were preferably oriented transversally (Fig. 10F). On average, the transversal distance between two Purkinje cells showing complex spike co-modulation was 0.23 ± 0.15 mm, while that of two simple spike co-modulating cells was 0.68 ± 0.37 mm \( (P = 0.0002, \) Student’s \( t \) test). Thus, while the complex spike co-modulation is organized in line with the sagittal projection zones of the inferior olive (Ruigrok, 2010), that of the simple spike co-modulation follows, at least in the anaesthetised state, roughly the trajectories of the parallel fibres (Braitenberg & Atwood, 1958).

To compare complex spike synchrony during rest and during whisker movements, we constructed joint peri-stimulus histograms (Fig. 10A). The dots on the 45 deg line reflect true synchrony of the two Purkinje cells. The histogram of this 45 deg line, after correction for changes in the complex spike frequency, shows changes in synchrony over time (Fig. 10A, bottom left). It turned out that the complex spike synchrony is constant throughout the recording, irrespective of the whisker movement. Consistently, cross-correlograms of traces including and excluding the periods of whisker movement were nearly identical (Fig. 10B). Yet, we found in all eight strongly co-modulating Purkinje cell pairs that the synchrony index was slightly lower in the parts of recording excluding the whisker movements (Fig. 10C). Taken together, we conclude that Purkinje cells ‘listening’ to the same sensory input have a large chance to belong to the same functional ensemble of Purkinje cells, the location of which is not necessarily confined to a narrow anatomical band.

However, the sensory input itself does not, or only to a limited amount, acutely contribute to the complex spike synchrony.

**Discussion**

Although the rodent whisker system is widely used as a model system to study sensory input, functional data on whisker encoding by the cerebellum were virtually absent. We show here that mechanical stimulation of a single whisker elicits both complex spike and simple spike responses in cerebellar Purkinje cells. The complex spike responses differ from the simple spike responses with respect to their receptive fields. In addition, Purkinje cell pairs showing complex spike responses to stimulation of the same whisker(s) are predominantly oriented along the sagittal plane, whereas Purkinje cell pairs showing simple spike responses to stimulation of the same whisker(s) are mainly oriented along the transversal plane. In either case, synchrony is more prominent among Purkinje cell pairs responding to the same whisker than between other Purkinje cell pairs, indicating the existence of functional ensembles of Purkinje cells cooperating in sensory encoding.

**The impact of anaesthesia**

The use of anaesthesia can have profound effects on the physiology of Purkinje cells (e.g. see Schonewille et al. (2006a)). Indeed, the average firing rate in our hands was about 20–25% lower in the Purkinje cells recorded in anaesthetised mice compared to those recorded in awake mice. Yet, qualitatively, the Purkinje cell responses to mechanical stimulation of the whiskers were rather similar in both states. In total, whisker responses were more often found in Purkinje cells of awake mice than in those of anaesthetised mice, and the ‘awake’ responses also tended to be larger and longer lasting. However, these effects can probably largely be explained by the larger number of whiskers stimulated with the air puff stimulation in awake mice than with the piezo-stimulation in anaesthetised mice. It should be noted that, in anaesthetised mice, stimulating three whiskers instead of one also increased the prevalence of Purkinje cell responses considerably (Table 1). The use of anaesthesia enabled us to make
Figure 9. Synchronous firing in anaesthetised mice
A, raster plot showing the occurrence of complex spikes in 7 Purkinje cells recorded simultaneously. Several Purkinje cell pairs showed a marked correlation in complex spike firing. Two pairs have been highlighted here: PC1 vs. PC5 and PC4 vs. PC7. Complex spikes that occurred within 10 ms of each other are marked red or green, respectively. Complex spikes that occurred within 100 ms of each other are marked dark red and dark green, respectively.
B, relative locations of the recording electrodes used for the recordings in A. The inter-electrode distance is 305 μm (heart-to-heart) in the x- and y-direction. C, cross-correlogram of the complex spikes of PC1 vs. those of PC5 (2 ms bins). Inset: enlargement of the middle part (from −500 ms to +500 ms). The black line indicates the average number of complex spikes during the interval from −3 to −2 s. The green line indicates the threshold (mean + 5 S.D.). D, as for C but now for the pair PC4 vs. PC7. E, the synchrony indices for the two pairs shown in C and D and for the pair PC1 vs. PC3 (that did not show any correlation in complex spike firing).
F, in total, 295 pairs of Purkinje cells were compared. In 42% of these, the patterns of complex spike firing were significantly correlated. About half of these pairs showed synchronous firing within 10 ms, with a part showing complex spike synchrony within 5 or even within 2 ms. G, the timing of the maximal peak in the cross-correlogram (y-axis) increased with larger (Euclidean) distances between the two Purkinje cells of each pair. Black line: linear regression line. H, as for G but now for the difference in depth. Purkinje cells that are located at a deeper location tended to fire before more superficial Purkinje cells. *P < 0.05 (linear regression).
I, synchronous simple spike firing in a Purkinje cell.
Complex spike population coding

Whisker input can increase complex spike firing dramatically – in some cells by more than 2500%. Nevertheless, we found that on average only around 10% of the trials evoked a complex spike with a stimulus of mediocre strength. The incidence of complex spike responses could be modulated by direction, amplitude and speed of the whisker movement. Thus, although the complex spike response is in itself an all-or-none response, the chance of complex spike firing depends dramatically – in some cells by more than 2500%. Whisker input can increase complex spike firing in the sagittal plane. Thus, Purkinje cells are clustered in functional ensembles based on their complex spike firing pattern. Forming such functional ensembles greatly increases the reliability of sensory encoding (Ozden et al. 2009; Schultz et al. 2009).

Complex spike synchrony is controlled by olivary activity (Llinás & Sasaki, 1989; Blenkinsop & Lang, 2006; Marshall et al. 2007). The orientation of the climbing fibre projections in the sagittal plane (Voogd & Glickstein, 1998; Ruigrok, 2010) fits with our observation that functional ensembles of Purkinje cells are also largely oriented sagittally. Since also the output of Purkinje cells to the cerebellar nuclei is organized in sagittal zones, this complex synchrony may enhance the Purkinje cell impact on the neurons of the cerebellar nuclei (Gauck & Jaeger, 2000; Hoebeek et al. 2010).

Complex spike synchrony has been shown to be enhanced during motor activity, but not during sensory processing (Welsh et al. 1995; Welsh, 2002). In line with this, we found that the complex spike synchrony was not instantaneously enhanced following whisker stimulation, but rather reflected a continuous functional binding.

Fractured somatotopy and sagittal zones

Local field potential recordings in the granule cell layer have shown that the lobules crus 1 and crus 2 have a fractured somatotopy, implying that there are patches of granule cells responding to tactile and/or nociceptive stimuli to specific parts of the body (Eccles et al. 1972; Shambes et al. 1978; Bower & Woolston, 1983; Bower, 1997). In rats, the largest whisker projection area in the cerebellar cortex is located in crus 1, occupying the largest part of that folium. In addition, several smaller patches are situated in other folia, including crus 2 (Joseph et al. 1978; Shambes et al. 1978). Such a detailed somatotopic mapping has not been made at the climbing fibre level, but there is a large, although not complete, similarity in the receptive fields of the complex spikes of Purkinje cells and those of the underlying granule cells (Brown & Bower, 2001). This is in line with the finding that the projection areas of the mossy fibre and the climbing fibre are largely, but not completely, similar (Thach, 1967; Eccles et al. 1972; Garwicz. et al. 1998; Voogd et al. 2003). In general, while the climbing fibres terminate in well-defined sagittal zones (Armstrong et al. 1973a,b; Groenewegen & Voogd, 1977;
The peri-stimulus histograms of the two simultaneously recorded Purkinje cells showed a strong complex spike response upon whisker stimulation. The whisker movement is shown in green. The JPSTH of both showed a strong complex spike response upon whisker movement than during periods of rest.

Receptive field topography

Ekerot & Jörntell (2001) applied 'natural' stimulations to the cat forepaw and measured the receptive field in the Purkinje cells are also shown. The histogram in the lower left corner is the histogram over the bins on the 45 deg line in the JPSTH, normalized for the firing rate per bin. It can be seen that the degree of synchrony does not vary to a large extent over the period, which indicates that the complex spike synchrony is not significantly larger during whisker movement than during periods of rest. B, cross-correlograms of the Purkinje cell pair depicted in A. The green line is the cross-correlogram of the whole recording, the black line is constructed with the omission of the 500 ms following the onset of stimulation (see the black and blue lines in the histogram in the lower left corner in A). The y-axis has been corrected for the different absolute numbers of complex spikes for the two conditions. C, synchrony indices for the whole trace (green line) and for the trace without the 500 ms following movement onset (black line). For comparison, the brown line shows the synchrony indices after a random shuffling of the inter-spike intervals (ISIs). The data in the panels A, B and C originate from the same Purkinje cell pair. D, Purkinje cells that both show a strong modulation in complex spike firing (> 5 s.d. of baseline rate, 'strong co-modulation') have a strongly increased chance to fire in a correlated or even a synchronous manner. Purkinje cell pairs of which both Purkinje cells showed a significant complex spike response to stimulation of the same whisker(s) (> 3 s.d., but not both > 5 s.d., 'weak co-modulation') did show an increase in correlated firing, but hardly of truly synchronous firing. *P < 0.01 (as compared to fraction of non-co-modulating Purkinje cell pairs, Fisher's exact test). E, Purkinje cell pairs showing strong complex spike co-modulation were located in a sagittal band. Each line connects the surface-projected locations of two Purkinje cells showing strong complex spike co-modulation. F, as for E but for the simple spikes. Simple spike co-modulation is predominantly found along the transversal axis.

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C3 zone of the cerebellar cortex. They found that the receptive fields of the complex spike responses in Purkinje cells were largely similar to those of the mossy fibres terminating in the granular layer below these Purkinje cells. Thus, they concluded that the receptive fields of the mossy fibres and the climbing fibres were largely similar, confirming earlier reports (Thach, 1967; Eccles et al. 1972; Bower & Woolston, 1983; Garwicz et al. 1998). A substantial fraction of their Purkinje cells (36%) showed a decrease in simple spike firing following stimulation of the (centre of the) climbing fibre receptive field. This inhibition can probably be explained by the action of the molecular layer interneurons, which have a very similar receptive field as the climbing fibres (Ekerot & Jörntell, 2001). The importance of molecular layer interneurons is supported by the finding that cerebellar granule cells are largely silent during rest, but can be activated by high-frequency bursts of mossy fibre inputs, which in turn can be triggered by tactile input (Chadderton et al. 2004; Rancz et al. 2007). Because of the low firing rate of the granule cells during rest, it is unlikely that their activity can be further decreased by diminished mossy fibre input. Hence, the decrease in simple spike firing must have its origin within the cerebellar cortex, and the molecular layer interneurons seem to be the most likely cause of sensory-induced simple spike inhibition. Molecular layer interneurons receive similar inputs as the Purkinje cells in the same sagittal zone (Ekerot & Jörntell, 2001) and readily inhibit simple spike firing (Eccles et al. 1966; Häusser & Clark, 1997).

Remarkably, while the receptive fields of the mossy fibres and of the climbing fibres largely overlap with that of inhibited simple spike firing, increased simple spike firing could also be evoked by skin stimulation, but had a different receptive field (Ekerot & Jörntell, 2001). Finally, the receptive field size of the simple spike responses is subject to bidirectional plasticity (Jörntell & Ekerot, 2002). The topography of the Purkinje cell receptive fields on the scale of individual whiskers partially confirms that of cutaneous stimulations of the cat forepaw (see Ekerot & Jörntell, 2001). Whisker stimulation evoked both inhibitory and excitatory simple spike responses to tactile stimulation. As with forepaw stimulation, the receptive fields of the simple spike and complex spike responses of a given Purkinje cell could be different.

While the simple spike responses of a given Purkinje cell to forepaw stimulation were either inhibitory or excitatory, depending on the stimulation area, simple spike responses to whisker stimulation were often bi-phasic: an increase in simple spike firing was directly followed by a period of simple spike inhibition. The temporal sequence, first excitation, then inhibition, is in line with the putative role of molecular layer interneurons in causing the inhibition of simple spike firing, as discussed above. Another possible source of simple spike inhibition, the complex spike pause, was found to have only a limited effect (Fig. 3), making the involvement of molecular layer interneurons more likely. Because of the bi-phasic nature of simple spike responses in individual Purkinje cells to single whisker stimulation, the receptive fields of inhibitory and excitatory simple spike responses were closely related, and not separated as following forepaw stimulation. The receptive fields of the complex spike responses were independent of those of the simple spike responses, but the receptive fields of inhibitory and excitatory simple spike responses to single-whisker stimulation were highly related, suggesting that molecular layer interneurons indeed have a local action.

Simple spike responses occurred with different latencies. We discriminated between early (latency <20 ms) and late responses (latency >40 ms). In both cases, an excitatory response could be followed by an inhibitory one. A similar division between early and late responses to sensory stimulation has been reported previously, and it has been shown that late responses involve the cerebral cortex (Kennedy et al. 1966; Woolston et al. 1981; Morisette & Bower, 1996). Both the somatosensory cortex and the motor cortex may therefore be involved in the late response (Fig. 1A). Early responses, therefore, most probably reflect activity of the (direct) trigemino-cerebellar and the trigemino-ponto-cerebellar mossy fibre pathways (Fig. 1A). We found that early responses were primarily associated with single-whisker stimulation, and late responses by multiple-whisker stimulation, indicating that both pathways convey different information.

**Radial vs. beam hypothesis**

The hypothesis of fractured somatotopy has been linked to the radial hypothesis of granule cell action (Bower, 1997). Granule cell axons ascend to the molecular layer, where they bifurcate and form long, transverse parallel fibres (Ramón y Cajal, 1888; Braitenberg & Atwood, 1958; Brand et al. 1976). The radial hypothesis proposes that the ascending part of the axon is functionally (much) more relevant than the transverse parallel fibre beams (Llinás, 1982; Bower, 1997; Cohen & Yarom, 1998). This hypothesis is supported by the finding that the receptive fields of the complex spikes in Purkinje cells and those of the underlying granule cells are largely overlapping (Brown & Bower, 2001). Interestingly, we found that Purkinje cells of animals in the anaesthetised state show simple spike responses to stimulation of the same whisker(s) and simple spike synchrony, were predominantly oriented in the transversal plane (Fig. 10F), perpendicular to the Purkinje cells responding with complex spikes (Fig. 10E). These results remain to be confirmed in awake animals, but they raise the possibility that there are functional
ensembles of Purkinje cells oriented along the parallel fibre beams.

Our data strongly suggest that the input from a single whisker is not projected to a single spot in the cerebellar cortex, but spread over a larger area. This could facilitate the rapid integration of tactile input from specific areas into a larger sensory context, as suggested by Bower (1997). Thus, the cerebellar cortex acts differently to the cerebral cortex, where sensory input is first processed in a specific region, and integration with other sensory inputs occurs only at a later stage (Engel et al. 2001). In line with this, the receptive fields of the whiskers were dispersed over crus 1 and crus 2 and not grouped as in the barrel cortex (Woolsey & Van der Loos, 1970; Petersen, 2007).

Conclusion

To our knowledge, this is the first study on the encoding of somatosensory input to Purkinje cells using such well-defined minimal receptive fields as single whiskers. From our study, it has become clear that complex spike responses have a very small receptive field, consisting of only one or a few whiskers, while the simple spike firing pattern of a given Purkinje cell is modulated by several whiskers. The simple spike receptive field lacks a clear ordering. Furthermore, the receptive fields of nearby Purkinje cells are apparently completely intermingled. We propose that this ordering, together with the previously described fractured somatotopy (Shambes et al. 1978; Bower, 1997), facilitates the integration of sensory information from various sources. In addition, we provide evidence that, at least in the anaesthetised state, Purkinje cells cooperate in different functional ensembles, based on shared receptive fields and synchronous firing. In line with the cerebellar anatomy, complex spike responses are oriented in the sagittal plane and simple spike responses in the transversal plane. This implies that individual Purkinje cells are members of at least two functional ensembles, further facilitating the rapid integration of diverse sensory inputs. In view of the impact of the cerebellum on motor output, we expect that such rapid integration of sensory input will contribute strongly to the integration of sensory and motor commands and thus to the motor output of the animals.

References


Acknowledgements

The authors wish to thank Alex Brouwer, Hans van den Burg, Wim Groeneveld and Erika Sabel-Goedknegt for their excellent technical support, and Drs John Simpson and Christiaan de Kock for the fruitful discussions. Our research was supported by the Dutch Organization for Medical Sciences (C.I.D.Z.), Life Sciences (C.I.D.Z.), Senter (Neuro-Biik, C.I.D.Z.), Prinses Beatrix Fonds (C.I.D.Z.), and the SENSOPAC, CEREBNET, and C7 programs of the European Community (C.I.D.Z.).
3.2

Effects of cerebellar inhibitory input on post climbing-fiber-pause activity in Purkinje neurons during whisker stimulation


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In contrast to the well-studied excitatory input to cerebellar Purkinje cells, the impact of inhibitory input to Purkinje cells is still under some debate. Olivary input to the cerebellum initiates complex spikes at the Purkinje cell, while mossy fiber input from the pontine nuclei modulates simple spikes. Although Purkinje neurons are typically posited to communicate via smoothly modulated firing rates, they may also use temporal patterns of spikes and abrupt silent periods releasing downstream inhibition. If the cerebellum implements a functionally relevant spatiotemporal code, the interplay between complex spike firing, simple spike pauses after the complex spike (climbing fiber pause), and simple spike firing may convey functional attributes not yet understood. We aimed to investigate how the absence of molecular layer interneuron input affected post-climbing fiber pause – simple spike responses during whisker stimulation. To this end, we used extracellular recordings to measure cellular activity of Purkinje cells in awake PC-Δγ2 mice, in which GABAA receptor – mediated synaptic inhibition is selectively removed from Purkinje cells, while stimulating the C-row of the ipsilateral whisker field.

We found significant changes in post complex-spike, simple-spike activity following whisker stimulation in wild-type mice that we did not find in PC-Δγ2 mice. The fraction of cells showing post-complex spike facilitation was significantly reduced during whisker stimulation in wild-types, but not in mutants. Whisker stimulation reduced peak responses in wild-type but not in mutants and differences in simple spike frequency in stimulated cells and non-stimulated cells were also greater in controls. These results suggest that blocking feed-forward inhibition from molecular layer interneurons in a genetically chronic fashion does not only affect the dynamics of inhibition, presumably due to compensatory mechanisms, but also excitation. We propose that molecular layer interneurons allow for more flexible firing components during peripheral stimulation adding to a growing body of evidence indicating a role for feed-forward inhibition in plasticity and learning.
Introduction

Input from the inferior olive to the cerebellar cortex directly contacts Purkinje cells via the climbing fibers. Climbing fiber activity causes stereotypical “complex spike” activity in postsynaptic Purkinje cells. Electrototically coupled clusters of olivary neurons project to Purkinje cells located in a sagittal zone. In addition, Purkinje cells are innervated by transversely organized beams of parallel fibers whose activity modulates the intrinsically generated simple spikes. The task for molecular layer interneuron inhibition might then be to modulate beams of parallel fiber input, inhibiting off-beam areas creating a sharper contrast for on-beam areas of excitation (Cohen and Yarom, 2000, Gao et al., 2006). These interneurons provide feed-forward inhibition crucial to the Purkinje cell, hence acute deletion or inhibition of this input can increase intrinsic simple spike firing of the Purkinje cell but also disrupt not only spatial, but temporal precision of simple spike output (De Zeeuw et al., 2011) but see also (Bower, 2010). Molecular layer interneurons make contact with, and therefore can be excited by, mossy fibers, parallel fibers, or the climbing fiber while also receiving inhibitory inputs from neighboring interneurons (Hausser and Clark, 1997).

As a result, the dynamic firing of the Purkinje cell exhibits a convolution of its many inputs, both direct and recurrent. For example, complex spikes are followed by a pause in simple spike activity, also known as the climbing fiber pause. A complex spike is caused initially by the opening of postsynaptic AMPA receptors that activate voltage-gated sodium channels, followed by the opening of potassium and calcium channels, which is then followed by after-hyperpolarization (Schmolesky et al., 2002). Just as climbing fiber input affects simple spike patterns, mossy fiber activity can indirectly modify climbing fiber activity through GABAergic feedback from the cerebellar nuclei to the inferior olive.

So, simple spike patterns can be modified by intrinsic activity of the cell itself and by input from the ascending granule axons, the climbing fiber, parallel fibers and the molecular layer interneurons.

Cerebellar Purkinje cells converge onto and inhibit cerebellar nuclei cells. Therefore the dynamic firing of complex spikes, simple spikes, and their periods of silence in one Purkinje cell, forms a dynamic interplay relevant to both spatial and temporal patterns of downstream activation. Furthermore, cerebellar interneurons have a short membrane time-constant and fast rise and decay times that can create fast and precisely timed spike outputs to inhibit Purkinje cells (Jorntell et al., 2010).

Reduction in molecular layer interneuron input has already been shown to lower variations in simple spike inter-spike intervals and parallel-fiber activation evoked fewer spikes consistent with reduced parallel fiber excitatory input. These firing effects were also consistent with reduced adaptation during vestibulo-ocular reflex and consolidation in gain adaptation (Wulff et al., 2009).
Previously we have shown that Purkinje cells exhibit both a complex spike response to air puff whisker stimulation and simple spike excitation and inhibition in response to air puff stimulation to the whiskers (Bosman et al., 2010). We showed that the simple spike inhibition by sensory stimulation could be independent from the complex spike, therefore, it may be created by other mechanisms than the complex spike pause (Bosman et al., 2010). However, there is also evidence in anesthetized mice that air puff stimulus to the whiskers does not initiate complex spike responses, and that inhibition due to whisker stimulation is strictly caused by molecular layer interneurons (Chu et al., 2011).

Therefore, we aimed to investigate how the absence of molecular layer interneuron input affects post-complex spike - simple spike responses during whisker stimulation in the awake mouse (De Zeeuw et al., 2011). We used rigorous categorization methods for facilitation (increased simple spike firing following a complex spike) and suppression (decreased simple spike firing following a complex spike) response types. There is a significant decrease in the ratio of cells that exhibit facilitation during whisker stimulation in wild-type animals, but not in mutants. The average peak facilitation in cells that do show this response is significantly less in wild-type animals during stimulation, but not in mutants. Furthermore, simple spike frequency is significantly higher for cells that show facilitation in wild-type animals during both non-stimulation and stimulation, but not in mutants. We propose that cerebellar molecular layer interneurons assist in flexible firing components of the Purkinje cell adding to a growing body of evidence for a role of feed-forward inhibition involved in plasticity.

Methods

A total of and 8 adult, PCΔγ2 mutant mice (85 cells) and 7 adult, wild type littermates (55 cells) were used for this study. All animals were head-fixed and awake during electrophysiological recordings. All experimental procedures were approved in advance by the local animal welfare committee, as required by Dutch law.

Head post surgery

Mice were initially anesthetized with isoflurane (4% V/V in O₂), and maintained under anesthesia with 2% isoflurane during the surgery. Eyes were covered with a layer of petroleum jelly (Alcon Duratears, Eye Ointment, Belgium). Core body temperature was monitored and maintained at 37°C. 25 μl of rimadyl was injected subcutaneously 15 minutes prior to the surgery. Fur was removed from the dorsal surface of the skull. An incision was applied to the skin above the skull from between the eyes to 1.5 cm in caudal direction. A few drops of lidocaine (10 μl, 0.5%) were applied to the exposed region. The periosteum was scratched off with a micro curette. The exposed skull area was thoroughly cleaned and dried with air. Two drops of bonding agent (OptiBond, all in one, Kerr, Italy) were applied on the skull
surface, followed by 30 seconds of UV light. A thin layer of Charisma (Heraeus, Germany) was applied to the skull. A magnetic pedestal was implanted by attaching it to the Charisma and fixed with 30 seconds UV light. The position of pedestal was centered on bregma. During the habituation sessions, the magnetic pedestal was attached to a metal part in front of the restrainer that fixed the animal’s head in an appropriate position. After 2 days of recovery, the mice began habituation sessions. Habituation sessions consisted of 2-3 sessions of one hour in the head restrained setup while place in the faraday cage. On the day of the experiment, the mice were transiently anesthetized using isoflurane and a craniotomy was made above crus 1 and crus 2 of the right hemisphere. Recordings started at least 60 minutes after the mouse was fully awake and actively whisking.

Electrophysiological Extracellular Recordings

Recording electrodes (2-5 MΩ) were made from quartz-coated platinum-tungsten fiber (outer diameter, 80 µm; Thomas recording, Giessen, Germany) according to the design of Eckhorn (Mountcastle et al. 1991). The electrodes were placed in a 8×4 matrix with an inter-electrode distance of 305 µm (Fig. 1B). Each electrode was inserted individually in the vicinity of a Purkinje cell in crus 1, crus 2 or vermis with a depth of 200-2500 µm. Up to 24 electrodes were used simultaneously. The electrophysiological signal was digitized at 12-25 kHz, using a 3-6000 Hz band-pass filter, amplified and stored using a RZ2 multi-channel workstation (Tucker-Davis Technologies, Alachua, FL, USA). The recording consisted of 2- 4 blocks of 20 to 30 minutes each. The first block consisted of only spontaneous whisking while the following blocks consisted of either pseudo-randomized interval or fixed interval (4 seconds) air puff stimulation.

Whisker Stimulation from Air Puffs

Whisker stimulation was executed by repeated air puffs. The air puffs (~300 mbar, 5 ms) were generated using a MPPI-2 pressure injector (Applied Scientific Instrumentation, Eugene, OR) and delivered by a small tube (2 mm diameter), placed approximately 3 cm above the whiskers at a 50° angle (with respect to the body axis) to deflect the whiskers in the caudal direction. The air stream was directed away from the face, in order to avoid stimulation of the eye and/or other facial structures. Repeated air puffs were either delivered with a pseudorandom (R) or fixed (F) inter-puff interval. (F) inter-puff intervals were 4 seconds while (R) interpuff intervals had 10 different intervals averaging 4 seconds over 10 puffs.

Electrophysiological data analysis.

Neuronal recordings were analysed online using OpenEx software suite (Tucker-Davis Technologies), and later re-analysed offline using SpikeTrain (Neurasmus B.V., Rotterdam, The Netherlands) running under Matlab (Mathworks, MA, USA). Putative events were first
detected using an amplitude threshold. Next, all putative events were superimposed and a waveform-analysis under full visual control was used to select true action potentials. For each Purkinje cell, we constructed a complex spike-triggered simple spike histogram to test for the presence of a pause in simple spike firing following each complex spike (Granit & Phillips, 1956; Bell & Grimm, 1969). Only recordings that had a complex spike pause of at least several milliseconds (complete pause > 4 ms) were considered to be “single-units” and only these recordings that showed stable amplitude for a minimum duration of 120 seconds were used for further analysis. When, during a recording, the signal-to-noise ratio decreased, analysis was stopped when there was either no longer a complex spike pause, or when the signal-to-noise ratio no longer allowed reliable detection of spikes.

Statistical significance of peri-stimulus time histograms was evaluated by defining a control period, during which we calculated the average and the 99% confidence interval. A response or correlation was considered “significant” when it exceeded the threshold during at least one bin of the “responsive period”, as defined below. In order to control for “false positives”, we searched for threshold crossings during a time interval, equally long as the responsive period, during the control period. Under most conditions, the 99% confidence interval was not associated with any “false positives”, but for a subset of experimental groups, the 99% confidence interval was not satisfactory and we adjusted the threshold as described below. For each cell the firing rate, coefficient of variation (CV), mean CV2, average inter-spike interval (ISI). CV is the standard deviation of ISI divided by the mean. The CV2 is calculated as the mean of \(2(\text{ISI}_{n+1} - \text{ISI}_n)/(\text{ISI}_{n+1} + \text{ISI}_n)\). These are measures of regularity of firing where CV reflects the entire recording of the mean, and the mean CV2 reflects regularity of adjacent intervals, and therefore is calculated on smaller timescales.

**Complex spike Pause, Suppression, Facilitation**

The complex spike triggered SS PSTHs (1ms time bins) were convolved with a gaussian kernel (halfwidth 11 ms) and normalized by the baseline firing rate per cell (defined as the average firing rate in the time window (1000ms - 200 ms before complex spike)). Climbing-fiber pause duration was calculated as the time for simple spike activity to return to 50% of baseline firing rate. Significant facilitation was considered as 3 standard deviations (calculated in the time window 1000ms - 200ms before complex spike) above baseline for at least five consecutive time bins. Significant suppression was considered as 3 standard deviations below baseline firing rate for at least 5 consecutive time bins.

**Post Climbing-Fiber Pause Response Types**

Simple spike responses to complex spikes are variable across cells. There may be more than one mechanism involved in facilitation and suppression. Therefore, based upon the criteria above for establishing cells with suppression or facilitation, we further categorized cells into
4 groups: cells with no suppression and no facilitation, cells with suppression only, cells with facilitation only, and cells with facilitation followed by suppression (See figure 1).

Figure 1: Response categories: Exemplary complex spike triggered peri-stimulus time histograms of cells with different response types. Horizontal hashed green lines indicate baseline and +/- 3 standard deviations. Vertical green lines indicate start or end time of responses. Light blue lines indicate duration of facilitation. Pink lines indicate duration of suppression. Dark blue lines indicate the fit during facilitation and orange lines indicate the fit during suppression using a least squares fit with an exponential function.

Results:

Whisker Stimulation and Spontaneous Whisking Results: Electrophysiology

Average simple spike and complex spike frequency of all cells are not different during spontaneous whisking in both WT and PCΔγ2

We found that average complex spike (CS) frequency and simple spike (SS) frequency of WT and PCΔγ2 during spontaneous whisking was not significantly different (WT CS freq = 1.4Hz ± 0.3, n =33; PCΔγ2 CS freq = 1.34Hz ± 0.09, n = 23; p = 0.73 (t-test); WT SS freq = 69.5Hz ± 3.84, n = 33; PCΔγ2 SS freq = 66.0 Hz ± 5.84, n = 23; p = 0.61 (t-test));

Average simple spike frequency of all cells are not significantly different during stimulation in both WT and PCΔγ2

We found that average simple spike frequency was lower in WT and PCΔγ2 cells during whisker stimulation (WT no stim n = 34, stim n=50; PCΔγ2 no stim n = 24, stim n = 31) but this was not significant (no stim: WT: 70 ± 3.8 Hz; PCΔγ2: 65.3 ±5.5 Hz; stim: WT: 64.8 ± 3.2 Hz PCΔγ2: 61.4 ± 4 Hz; p = 0.14 (t-test)).
The ratio of WT cells showing facilitation during stimulation is significantly reduced, but not in PCΔγ2.

We found that the ratio of WT facilitation cells during stimulation was significantly reduced, but not in PCΔγ2. (WT no stim: n = 27/33; stim: n = 23/50 p = 0.011 Fisher’s exact test; PCΔγ2 no stim: n = 8/24; PCΔγ2 stim: n = 16/31; p = 0.27 Fisher’s exact test; see figure 2A).

The peak amplitude of facilitation cells in WT is significantly decreased during stimulation but not in PCΔγ2.

We found that cells that did show facilitation during both non-stimulation and stimulation, average peak facilitation amplitude above baseline was significantly less in WT but not in PCΔγ2. (WT no stim: 52.3 ± 4.2%; stim: 39.5 ± 3.7%; p = 0.02 (t-test); PCΔγ2 no stim: 39.1 ± 0.9%, stim: 39.0 ± 3.7%; p = 0.99 (t-test); see figure 2B).

Figure 2: (A) The ratio of WT facilitation cells is reduced during stimulation while MT facilitation cells are not (left panel). (B) WT facilitation cells show a decrease in peak response while MT do not (right panel).

During spontaneous whisking, average simple spike frequency is greater in WT facilitation cells than WT non-facilitation cells, but not in PCΔγ2.

During spontaneous whisking, average simple spike frequency was significantly greater in WT facilitation (75.1 ± 3.7 Hz) (n = 5) cells than in non-facilitation (44.1 ± 7.4 Hz) (n = 20) cells; p = 0.006 (t-test; bonferroni correction), but not in MT facilitation cells (67.6 ± 7.9 Hz) (n = 14) vs. non-facilitation (63.4 ± 8.0 Hz) (n = 8) cells; p = 0.72 (t-test) (see figure 3A).

During whisker stimulation, average simple spike frequency is greater in WT facilitation cells than WT non-facilitation cells, but not in PCΔγ2.

During whisker stimulation, average simple spike frequency was significantly greater in WT facilitation (72.8 ± 4.3 Hz) (n = 24) cells than in non-facilitation (57.4 ± 4.1 Hz) (n = 26)
cells; \( p = 0.003 \) (ANOVA); \( p = 0.013 \) (t-test), but not in MT facilitation cells (60.0 ± 5.1 Hz) (\( n = 14 \)) vs. non-facilitation cells (62.6 ± 6.2 Hz) (\( n = 17 \)) \( p = 0.75 \) (t-test) (see figure 3B).

Figure 3: (A) WT cells show greater simple spike frequency in facilitation cells than in non-facilitation cells during spontaneous whisking (A) and whisker stimulation (B). Lighter colors indicate average firing frequency from non-facilitation cells and darker colors indicate average firing frequency from facilitation cells.

Discussion

Complex spikes are followed by a pause in simple spike activity (Bloedel and Roberts, 1969), (Bloedel and Roberts, 1971) however whether there is a functional role of the simple spike activity following the climbing fiber pause in Purkinje cell firing is still unclear.

We found that spiking changes of Purkinje neurons after a climbing fiber pause during whisker stimulation activity occurred more often and was more extreme in WT mice than in mutant mice lacking GABA\(_A\) receptor - mediated synaptic inhibition.

Cerebellar cortex molecular layer interneurons are organized in beams and it has been argued that they play a role in sculpting the spatiotemporal output of the cerebellar cortex by suppressing activity, thereby releasing inhibition on cerebellar nuclei cells (Wulff et al., 2009). However there is evidence that climbing fiber activation also causes inhibition on Purkinje cells (Mathews et al., 2012). Furthermore, there are claims that pauses in simple spike activity do not represent additional information about sensory events (Cao et al., 2012). However, this study excluded pauses following complex spikes because there was no correlation found with the measured behavior (licking and breathing) (Bryant et al., 2010, Cao et al., 2012). The data shown here points to the notion that not only may sensory events effect spike rate modulation but molecular layer interneuron input may not be the only player in shaping Purkinje cell firing patterns. There is evidence that neighboring climbing fibers within the same microzone can fire in synchrony and this type of input may provide
a mechanism by which clusters of local interneurons within the same microzone can also become synchronized (Jorntell et al., 2010). Therefore, we aimed to investigate the relative contribution to inhibitory input on simple spike firing during and after the complex spike, both in the presence and absence of whisker stimulation. We propose that molecular layer interneurons allow for more flexible firing components during peripheral stimulation adding to a growing body of evidence indicating a role for feed-forward inhibition in plasticity and learning.
References


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3.3

Cerebellar plasticity and learning an object localization task with the use of whiskers


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Object localization with the use of whisker detection requires activation and plasticity of the somatosensory and motor cortex. These parts of the cerebral cortex receive strong projections from the cerebellum via its routes through the thalamus, but it is unclear whether and to what extent cerebellar processing may contribute to such a sensorimotor task. Here, we subjected cerebellar Purkinje cell-specific PP2B knock out (L7-PP2B) mice, which suffer from impaired intrinsic plasticity in their Purkinje cells and long-term potentiation (LTP) at their parallel fiber-to-Purkinje cell synapses, to an object localization task. Water deprived animals had to learn to detect the location of an object with the use of their whiskers, and based upon this location they were trained to lick water within a particular period (“go” trial) or refrain from licking (“no-go” trial). L7-PP2B mice were not ataxic and showed normal basic motor performance during whisking and licking, but learning the whisker-dependent object localization task was severely impaired compared to wild type littermates. Significantly less L7-PP2B mice were able to learn the task, those L7-PP2B mice that eventually learned the task made unstable progress, were significantly slower in the learning process, and showed deficiencies in temporal tuning, and these differences became greater as the response window became narrower. We conclude that cerebellar processing and potentiation in particular can contribute to learning a whisker-dependent object localization task when timing is relevant. This study points towards a relevant role of cerebello-cerebral interaction in a sophisticated cognitive task requiring strict temporal processing.
Introduction

Active touch by mystacial vibrissae forms a major source of sensory information for rodents (Carvell and Simons, 1990; Hartmann, 2009). Head-fixed mice can be trained to exploit such active exploration to associate the position of a stimulation bar in their whisker field with the availability of a water reward (O’Connor et al., 2010b). Whisker-based object localization has been shown to involve correlated neuronal activity in the barrel cortex (S1) and the whisker motor cortex (M1) (Xu et al., 2012). However, it is unclear to what extent other brain regions also contribute to such tasks. Given the numerous brain regions involved in whisker control and given their intricate connections (Bosman et al., 2011; Kleinfeld and Deschênes, 2011), one may well expect other areas to also play a role in whisker-based object localization. Here we focus on the cerebellum, a brain region important for sensorimotor integration, central to the whisker system, and required for procedural learning and accurate timing of fine movements (Grodd et al., 2001; Bosman et al., 2010; De Zeeuw et al., 2011).

Purkinje cells form the sole output neurons of the cerebellar cortex. Their activity depends on both synaptic and intrinsic plasticity (Hansel et al., 2001; Ito, 2001; Gao et al., 2012). In the absence of calmodulin-activated protein phosphatase 2B (PP2B) both enhancement of intrinsic excitability of Purkinje cells and long-term potentiation (LTP) at the parallel fiber-to-Purkinje cell synapses are impaired, while the regularity of their simple spike firing is increased (Schonewille et al., 2010). Purkinje cell-specific PP2B knock-out (L7-PP2B) mice show deficits in motor learning and consolidation, as demonstrated during adaptation of the vestibulo-ocular reflex and eyeblink conditioning (Schonewille et al., 2010). To date, of all currently available cell-specific cerebellar mouse mutants that are not ataxic the L7-PP2B mutant shows the clearest deficits in procedural learning (De Zeeuw et al., 2011; Gao et al., 2012). Yet, when subjected to standard non-motor tasks such as the regular Morris water maze, fear conditioning or social interaction task in which no fine temporal control is required, the L7-PP2B mutants do not show abnormal performance (Galliano et al., 2013).

The primary objective of this study was to investigate whether, and if so to what extent, potentiation of intrinsic activity and synaptic strength in the cerebellum is required for a localization task in which the response has to be given within an allotted response period following the insertion of a bar into the whisker field. To this end we tested L7-PP2B mice using a modified version of the object localization task introduced by O’Connor et al. (2010b) so as to further tighten the temporal constraints. We demonstrate that L7-PP2B mutants are severely impaired in learning this whisker-based object localization task showing for the first time that this learning task can depend in part on plasticity and/or processing in the cerebellum when timing is relevant.
Materials and Methods

The generation of mice lacking functional PP2B in their Purkinje cells has been described previously (Schonewille et al., 2010). Briefly, we used crossings of mice in which the gene for the regulatory subunit (CNB1) of PP2B was flanked by loxP sites (Zeng et al., 2001) with transgenic mice expressing Cre under control of the L7 promoter (Barski et al., 2000). L7-Cre\textsuperscript{+/-}-cnb1\textsuperscript{f/f} mice (“L7-PP2B mice”) were compared with littermate controls (“WT mice”) consisting of L7-Cre\textsuperscript{-/-}-cnb1\textsuperscript{f/f}, L7-Cre\textsuperscript{-/-}-cnb1\textsuperscript{+/-}, and L7-Cre\textsuperscript{+/-}-cnb1\textsuperscript{+/-} mice. All experimental procedures were approved by the institutional animal welfare committee as required by Dutch law.

Licking behavior

Since licking behavior was used as the behavioral read out of the localization task (see below), we first assessed the overall performance during baseline licking in 5 L7-PP2B mice and 6 WT littermates of either sex of approximately 12 weeks old. Baseline licking was measured in the home-cages of naïve mice by measuring threshold crossings in the junction potential between an aluminum floor plate and the spout of a normal drinking bottle with the use of an AD converter operating at a sample rate of 6 kHz (RZ2, Tucker-Davis Technologies, Alachua, FL) (Fig. 1A). Our experimental design was based on that of Hayar et al. (2006). Since mice normally lick very sparsely, they were deprived of water for 20 h prior to the period of experimental observation, which lasted approximately 1 h. We added 0.5 M sucrose to the drinking water to further increase the motivation to lick rhythmically (cf. Yamamoto et al., 1982). We restricted our analysis of baseline performance to bouts of rhythmic licking, which were defined by the occurrence of at least two licks within 175 ms; the beginning and the end of a licking bout were identified by preceding and consecutive periods during which no licks occurred for at least 175 ms (Fig. 1A). Licking during the training paradigm was detected by laser beam crossings. In order to avoid double detections, we used a dead time of 20 ms.

Auto-correlograms with a bin width of 5 ms were made of the lick times in the home cage as well as during the association task and the object localization task (see below). Side peaks were normalized to the center peak and detected as local maxima. The amplitude of these first side peaks was considered to be the strength of the rhythmicity. Rhythmic licking predominantly occurred at frequencies between 6 and 10 Hz. Further quantitative analysis was done in this frequency band. Licking was considered to be rhythmic if the first side peak exceeded the average + 3 SD of the period between 1000 and 800 ms before each lick.

Whisking behavior

As whisking behavior was used as the critical sensory detection mechanism for the localization task (see below), we also assessed the overall performance during free whisking in 4 adult L7-PP2B and 4 WT female mice (Fig. 1E-I). To this end we made high-speed
videos of spontaneous whisker movements in head-restrained mice (full frame rate between 600 and 1000 Hz; A504k camera, Basler Vision Technologies, Ahrensburg, Germany) using a red LED panel ($\lambda = 640$ nm) as backlight. In addition, videos were made during selected sessions of the training paradigm (see below; Figs. 2-4). The latter videos were recorded with a full frame rate of 160 Hz (piA640-210gm camera, Basler Vision Technologies) and infrared lighting to avoid vision ($\lambda > 900$ nm).

In order to establish the periods during which the mice actively moved their whiskers we estimated whisker motion using the BlockMatcher function in Matlab (Mathworks, Natick, MA). First, we selected a rectangular region of interest containing the proximal part of the whiskers. This region was sliced into a grid with rectangular blocks. Across contiguous frames, each block was transformed by a rotation and translation, such that the distance between the blocks in consecutive frames was minimized. The whisker motion was calculated as the Pythagorean addition of the translation and the rotation of all blocks (Fig. 1E).

The extent of whisker movements was further illustrated using either minimum or standard deviation (SD) projection plots of video fragments (ImageJ, NIH). During free whisking we made video fragments of single whisker bouts and constructed minimum projection plots to visualize the area that had been covered by the whiskers. During the training paradigms, each video fragment reflected the activity during a single response window (RW; see below). Here we used pseudo-colored SD projection plots to illustrate the whisker movements.

We calculated the whisking frequency by measuring the duration of 3 bouts of 2-4 whisks ($>10^\circ$ or more) using the 0.6-1.0 kHz videos. Movements of individual whiskers (of the 2$^{nd}$ arc) were tracked manually by detecting crossing of a vertical line at approximately 2.5 cm lateral to the skin and from these values the whisker angles were estimated. The amplitude was taken as the maximal difference in angle during a period of 400 ms.

**Habituation and association stage**

We prepared 14 female L7-PP2B mice and 16 female littermate controls, all of which were 20-25 weeks of age and carried a body weight of 22-25 g, for experimental testing. These mice received a magnetic pedestal that was attached to the skull above bregma using Optibond adhesive (Kerr Corporation, Orange, CA) under isoflurane anesthesia (2-4% V/V in O$_2$). Post-surgical pain was treated with 5 mg/kg carprofen (Pfizer, New York, NY) and 5 mg lidocaine (Braun, Meisingen, Germany). After two days of recovery, mice were put on water restriction (1 ml/day), while food was available ad libitum. On the fourth, fifth and sixth day of water restriction mice were put in a head-fixed position using the magnetic pedestal and habituated to the experimental set-up for one 15 min session per day. During these sessions water drops (~20 μl/lick) were triggered upon breaking the laser beam of the lick port. The mice did not receive extra water after the habituation sessions.

Upon completion of the habituation phase, the mice progressed to the association task to ensure that the L7-PP2B mutants had the same level of motor performance as the WT mice at
the onset of localization training. In this respect our protocol deviated from that in the study by O’Connor et al. (2010b), which was designed to describe correlates with cerebral cortical activity rather than to compare cerebellar phenotypes. During the association task the mice learned to associate the rising of a bar (~1 mm diameter) into their right whisker field with the availability of water at the lick-port. The association trials started with a horizontal movement below the reach of their whiskers (lasting for 2 s) of the stimulation bar, followed by a vertical movement (lasting for 850 ms) that placed the bar inside the whisker field (approximately 5 mm posterior and 10 mm lateral to the tip of the nose) (Fig. 2A; see also Movie 1). Once the bar reached its highest position, the water reward became available through the lick-port in that a drop of water was provided as soon as the mouse licked. This situation lasted for 2000 ms (i.e. the RW), after which the water supply was stopped, all remaining water was sucked out of the lick-port and the bar moved downwards returning to its starting position via the same route and at the same speed. When the animal did not respond to the stimulation during the RW, the next trial was postponed by an extra 3 s delaying the possibility of reinforcement. Licking outside the RW did not have any positive or negative consequences, except for the absence of water outside the RW. Each mouse was trained during one daily session consisting of 100 trials. The association task was completed as soon as a mouse licked within the RW in at least 80% of the trials for at least two consecutive sessions. To minimize visual cues, the entire task took place in complete darkness, except for some sessions in which we made a video of the whisker movements using infrared illumination; these videos were recorded with a full frame rate of 160 Hz using infrared lighting at \( \lambda > 900 \) nm.

**Localization learning**

Following completion of the association task mice continued with the object localization task consisting of “go” and “no-go” trials on the following day. During a go trial, the stimulation bar moved horizontally in the caudal direction from the neutral position below the right whisker field to approximately 5 mm posterior to the nose and then vertically into the whisker field as described above (see video sections in online material). During a no-go trial the stimulation bar moved horizontally in the rostral direction from the neutral position below the whisker field to approximately 5 mm anterior to the nose, and then vertically into the whisker field. The no-go position was outside the whisker field in rest, but could be reached during active whisking. The actual distance between the go and the no-go position depended on the size of the head and varied between 8 and 11 mm. A trial always began from and ended with the stimulation bar at rest in the neutral position, which was in the middle between the go and the no-go position, to ensure that the timing of go and no-go trials was identical. During rest at the neutral position and during the horizontal movements, the stimulation bar was well below reach of the whiskers. For both types of trials the RW started as soon as the vertical movement of the bar was completed, but only during the go trials the mice were rewarded with a drop of water when they licked the lick-port within the RW. The total duration of a trial
was approximately 6.2-7.2s, depending on the duration of the RW, followed by an inter-trial interval of 7 s in correct trials. An incorrect response (not licking) during a go trial resulted in an extra inter-trial interval of 3 s, whereas an erroneous response (licking) during a no-go trial resulted in an extra inter-trial interval of 8 s (Fig. 3A). Each (daily) session consisted of 100 pseudo-randomized trials (50% go and 50% no-go trials) or until the mouse discontinued licking, which was defined as not showing any responses for 10 consecutive trials. Once the mice had ≥ 80% correct during two consecutive sessions of the localization task with a RW of 2000 ms, the RW was decreased to 500 ms (via an intermediate step using a RW of 1000 ms) (Figs. 3-5). Mice that did not learn the 2000 ms localization task within 35 sessions were considered non-performers and they were not tested any further. For control, we cut all whiskers in 10 mice (under isoflurane anesthesia) following completion of the 500 ms localization task and we tested their performance again on the next day.

**Constructing learning trajectories**

For each session, we plotted the average hit rate and average false alarm rate of all mice per group (Fig. 6). To this end we calculated for each mouse and for each session the hit rate, i.e. the fraction of correct responses (licks) during the go trials relative to all go trials, and the false alarm rate, i.e. the fraction of incorrect responses (licks) during the no-go trials relative to all no-go trials. Linear regression lines were fitted to the group averages and the deviation from the linear regression was calculated as the least squared difference (SigmaPlot, Systat Software, Chicago, IL). The sensitivity index (d’) was calculated using the z transformations of the hit rate and the false alarm rates (d’= z(hit rate) - z(false alarm rate)), assuming a Gaussian distribution. The 80% correct level corresponded to a d’ score of approximately 1.7 (cf. Huber et al. 2012).

**Data analysis**

Summed learning curves were made for both the association stage and the object localization task by adding the performance for each mouse, so that the upper line represents the average performance for all mice in that group. Unless stated otherwise, data is represented as means ± SEM and statistical testing was performed using Student’s t test. For unrelated tests we used a level of significance of 5%. For repeated tests the level of significance was corrected using Bonferroni correction (a corr = α / n with α = 0.05 and n = number of tests). Where Bonferroni correction was applied, a corr is mentioned in the text.
Results

Licking in freely moving mice is comparable across genotype

Since we are using licks as the read-out parameter of learning capabilities during the object localization task, deficits in motor aspects of licking could in principle create a bias in the learning performance. Therefore, we first studied the licking behavior of WT and L7-PP2B mice in their home cages. Both WT (n = 6) and L7-PP2B mice (n = 5) licked during multiple periods. Such licking periods often consisted of a few bouts of uninterrupted licking, each of which consisted of a series of rhythmic licks (Fig. 1A-B). Neither the licking frequency (8.2 ± 0.3 Hz for WT versus 8.5 ± 0.4 Hz for L7-PP2B mice) nor the number of licks per bout (16.4 ± 4.9 and 13.5 ± 5.0) differed significantly among genotypes (p = 0.447 and p = 0.686, respectively) (Fig. 1C, D). In addition, the distributions of inter-lick intervals within bouts were similar between WT and L7-PP2B mice (p = 0.653; Kolmogorov-Smirnov test) (Fig. 1D). These data indicate that the baseline licking performance of freely moving L7-PP2B mice is intact.

Free whisking in head-restrained mice

Abnormal whisker use could be another confounding factor for our whisker-based object localization task. Therefore, we quantified the spontaneous whisker movements of 4 WT mice and 4 L7-PP2B littermates during recording sessions in which no whisker stimulation took place. All mice showed repetitive periods of whisking. During such movements, all mice were able to scan the whole area - from (almost) touching the fur in the most caudal position to (almost) touching the fur in the most rostral position (Fig. 1F). We quantified the movements of individual whiskers during bouts of rhythmic whisking. Within such bouts, neither the amplitude (WT: 61.4 ± 8.8°; L7-PP2B: 64.5 ± 7.7°; p = 0.698) nor the frequency (WT: 11.5 ± 0.7 Hz; L7-PP2B: 11.1 ± 1.2 Hz; p = 0.792) differed significantly between the two groups of mice (Fig. 1G-I). Thus we conclude that WT and L7-PP2B mice are similar in their range and frequency of free whisking.
Figure 1 - L7-PP2B mice do not have motor deficits preventing normal, rhythmic licking and whisking

A - A period of rhythmic licking in a freely moving L7-PP2B mouse. Licks can be seen as positive deflections of the junction potential between the spout of the drinking bottle and an aluminum floor plate in the home cage. This licking period consists of 3 individual licking bouts, each indicated with another color. B - Auto-correlograms of licking bouts in a WT (left) and a L7-PP2B mouse (right). The right panel is the auto-correlogram of the first licking bout depicted (in blue) in panel A. The center bin was removed to improve visibility. C - Both WT and L7-PP2B mice displayed short and long licking bouts with lick frequencies predominantly between 8 and 11 Hz. Shorter licking bouts tended to vary more in lick frequency than long bouts in both genotypes. The “red” licking bout shown in panels A-B is indicated by a larger symbol. D - Histograms of all inter-lick intervals within licking bouts showed similar distributions in WT and L7-PP2B mice, indicating that L7-PP2B had no motor deficits preventing them to lick rhythmically. The histograms were made with a bin size of 2 ms and the area under the plot was normalized to 100%. Inset: Average licking frequency per mouse (n = 6 WT and 5 L7-PP2B mice). E - We characterized periods of whisker movement using motion detection (see Methods). The top trace (orange) represents the angle of an individual whisker (of the 2nd arc) during a whisking bout. This trace was obtained by computer-aided tracking. 0° angle corresponds to perpendicular to the body axis (re = retraction; pro = protraction). The lower trace (black) shows the output of the motion detection. It can be seen that the motion detection algorithm can accurately detect periods of movement, but cannot be used for the quantification of whisker movement amplitudes.
Chapter 3

**F** - Minimum projection plots of single bouts of free whisking, each bout containing 3 whisks. The dark area indicates the space explored by the whiskers during this bout. It can be seen that both the WT and L7-PP2B mice spanned the whole area. The stimulation bar is visible in the picture, but it was well below the whisker field, so that it could not be reached by the mice during free whisking. In front of the mouse the lick port and the pedestal holder can be seen. **G** - Angular movements of a single whisker. Each plot shows an overlay of three bouts of rhythmic whisker movements, with each bout coming from another mouse. **H** - The amplitude of rhythmic whisker movements in the absence of whisker stimulation in L7-PP2B mice was comparable to that of WTs. **I** - as in **H**, but now revealing frequency.

**The association stage**

Since the frequency of licking can depend on the ease of access to water (Weijnen, 1998), we compared the licking behavior in head-restrained mice during the association task when water was available during the 2000 ms RW. Overall, the average number of licks per minute - as calculated over the first association session - was comparable between head-restrained WT \( n = 16 \) and L7-PP2B \( n = 14 \) mice (WT: 96 ± 18 licks/min; L7-PP2B: 120 ± 24 licks/min; \( p = 0.535 \); data not shown). Most mice (14 out of 16 (87.5%) WT and 14 out of 14 (100%) L7-PP2B mice; \( p = 0.485 \); Fisher’s exact test) licked rhythmically during the RW of the first association session. WT mice had a slightly different licking frequency, but the difference with L7-PP2B mice was not significant (f = 8.3 ± 0.1 Hz and 7.9 ± 0.1 Hz, respectively; \( p = 0.06 \)). The strength of the rhythmicity was similar (13.0 ± 0.8% and 11.8 ± 0.7%; \( p = 0.306 \)) (Fig. 2B). As the association training proceeded, rhythmic licking during the RW increased; at the end, the strength of the rhythmicity was 15.1 ± 1.0% and 14.3 ± 0.9% for WT and L7-PP2B mice, respectively (\( p = 0.576 \)). The frequency remained around 8 Hz for both groups (7.9 ± 0.1 Hz and 7.8 ± 0.1 Hz, respectively; \( p = 0.624 \)). Moreover, video analyses of the whisker movements showed that WT and L7-PP2B mice were both actively whisking during the association trials. Figure 2C shows an example of whisker movements in a video of a WT and L7-PP2B mouse during the first session of the association task. Both mice whisked actively and contacted the stimulation bar during the RW of the association task. Thus, as in naïve mice both WT and L7-PP2B mice had similar lick responses and active whisker exploration behavior during the RWs of the association task.

Finally, we analyzed the performance of the mice during the association training. We identified a trial as correct when a mouse licked within the RW independent from its activity outside the RW. On average both groups had similar percentages of correct trials during the first session when the rod was elevated inside the whisker field (WT: 39.4 ± 24.6%; L7-PP2B: 50.1 ± 24.2%; mean ± SD; \( p = 0.241 \)). In addition, the percentage of correct responses increased equally strong during the association task for both genotypes (last session: WT: 93.8 ± 5.4%; L7-PP2B: 91.4 ± 7.1%; mean ± SD; \( p = 0.238 \)). WT and L7-PP2B mice required a similar number of sessions to reach criterion (6.6 ± 3.6 and 6.4 ± 2.6 sessions, respectively; mean ± SD; \( p = 0.865 \)) (Fig. 2D). Thus, all mice - irrespective of their genotype - learned to lick during the RW of the association test at a similar pace.
Figure 2 - Mice learn to lick after feeling a stimulus bar in their whisker field

A - Learning paradigm. During the association task mice were only subjected to go trials and learned to lick following whisker contact with a metal bar (orange dot) within a 2000 ms response window (RW). Once the stimulation bar was moved horizontally, it moved vertically in the whisker field prior to the RW. This period, during which whisker contact became possible about half way of the vertical movement, is indicated in green. Correct responses triggered a water reward; incorrect responses postponed the next trial. B - Mice licked rhythmically during the RW of the association phase. During the association task the mice increased their licking rhythmicity as indicated by the increase of the amplitudes of the side peaks at 125 ms (corresponding to a dominant lick frequency of 8 Hz; cf. naïve mice in left panel and trained mice in right panel). The auto-correlograms were made with a bin size of 5 ms and normalized to the center peak (which is not shown to improve clarity). C - Standard deviation projection plot showing a representative example of whisker movement during the RW. The color bar at the bottom indicates the amount of movement (black = no change; white = maximal change). It can be seen that both mice moved their whiskers actively during the RW and touched the stimulus bar. D - Summed learning curves during the association phase. The inset shows the average number of sessions required to reach criterion.
General motor performance during localization training

Following completion of the association stage, during which mice only received go trials, they were subjected to the localization learning task, wherein they received both go and no-go trials. During the go trials the pole was positioned inside the whisker field (as in the association stage), whereas during the no-go trials the pole was raised just in front of their baseline whisker field so they could only detect the rod by means of active forward exploration (Fig. 3A). In contrast to the go trials when the mice were encouraged to lick, they had to withhold their licking during no-go trials in order to prevent a delay for the next trial postponing potential reinforcement.

First, we subjected the animals to trials with a RW of 2000 ms and we analyzed the licking pattern during the RWs of the first localization session. Licking was more rhythmic during the RW of go trials, when the mice received water, than during that of no-go trials, when the mice did not receive water (Fig. 3B). During go trials, the lick rhythm was again around 8 Hz for both genotypes (WT: 8.2 ± 0.1 Hz and L7-PP2B: 7.9 ± 0.1 Hz; p = 0.085) and it had a similar strength among genotypes (15.6 ± 1.0% and 14.4 ± 0.6%, respectively; p = 0.304; t test). During no-go trials, the lick rhythm was 8.1 ± 0.2 Hz for WT and 7.4 ± 0.2 Hz for mutants (p = 0.072), and the amplitude was 7.9 ± 1.0% and 7.4 ± 0.9%, respectively (p = 0.710). Likewise when we analyzed the whisker behavior during the localization task (from 5 WT and 5 L7-PP2B mice), we found active whisking during both go and no-go trials, irrespective of the genotype of the mouse (Fig. 3C-D). Moreover, in general the occurrence of lick responses of successful L7-PP2B mice within the 2000 ms RW progressed similarly to that in WT (compare development of licks in go and no-go trials in Fig. 3E for the first session with those in Fig. 3F for final session).

The mice that performed well during the 2000 ms object localization task were subsequently tested with the same test with a RW of 500 ms (Fig. 4A; Movie 1). During the 500 ms RW of the go trials, mice licked again rhythmically around 8 Hz (frequency: WT: 8.8 ± 0.4 Hz; L7-PP2B: 8.5 ± 0.3 Hz; p = 0.514; amplitude: WT: 10.3 ± 0.8%; L7-PP2B: 9.2 ± 1.0%; p = 0.385). Most likely related to the shorter presence of water (500 ms instead of 2 s), the occurrence of high-frequency tongue movements was relatively high in both WT and L7-PP2B mice, leading to a similar shape of the auto-correlograms (p = 0.795; Kolmogorov-Smirnov test; Fig. 4B). Licking during the RW of no-go trials was very sparse in both trained WT and mutant mice (Fig. 4E), precluding a meaningful quantification of lick rhythmicity during the no-go trials. During the last session of the 500 ms object localization task both WT and L7-PP2B mice whisked actively during the trials (Fig. 4C), but in comparison to naïve mice both genotypes whisked less during inter-trial intervals (cf. Fig. 3D and 4D).
Figure 3 - Motor behavior during the object localization task with a RW of 2000 ms

A - Learning paradigm. During the object localization task mice were subjected not only to go trials, but also to no-go trials. The mice had to learn to lick during the response window (RW) of the go, but not during that of the no-go trials. Once the stimulation bar was moved horizontally to the go or to the no-go position, it moved vertically into the whisker field prior to the RW. This period, during which whisker contact became possible about half way of the vertical movement, is indicated in green. Licks during the RW of go trials triggered a water reward; incorrect responses postponed the next trial. B - Mice licked rhythmically during the RW. Rhythmic licking was more prevalent during the go trials, when there was water, than during no-go trials, when there was no water. C - Standard deviation projection plot showing a representative example of whisker movement during the RW. It can be seen that both mice moved their whiskers actively during the RW and touched the stimulus bar, both in the go and in the no-go trials. D - Whisker motion plots for representative mice showing active whisking during the trials. Grey areas indicate the RW and green areas the periods of the preceding vertical movement. Go trials are indicated with a “G”, no-go trials with a “N”. Longer inter-trial intervals indicate incorrect responses. E - Raster plots of lick times showing the first 10 go (left) and no-go trials (right) of representative experiments during the first session of the 2000 ms object localization task. The two top panels show raster plots for a single individual per genotype. The bottom panel shows the histograms of the relative timing of the licks over all trials averaged for all performers. The green area (850 ms) refers to the interval during which the stimulation bar moved vertically, either into (go trials) or in front of (no-go trials) the whisker field. The grey area indicates the response window (2000 ms). F - Idem for the last session of the 2000 ms.
Figure 4 - Motor behavior during the object localization task with a RW of 500 ms

A - Learning paradigm. As described for Figure 3, once the stimulation bar was moved horizontally to the go or to the no-go position, it moved vertically in the whisker field prior to the RW. This period, during which whisker contact became possible about half way of the vertical movement, is indicated in green. Licks during the RW of go trials triggered a water reward; incorrect responses postponed the next trial.

B - Mice licked rhythmically during the RW of go trials of the last 500 ms object localization session. At this stage, the mice performed so well that licking during no-go trials was really sparse and that there were not enough licks during the RW of no-go trials to permit quantitative analysis.

C - Standard deviation projection plot showing a representative example of whisker movement during the RW. It can be seen that both mice moved their whiskers actively during the RW and touched the stimulus bar, both in the go and in the no-go trials.

D - Whisker motion plots for representative mice showing active whisking during the trials. Go trials are indicated with a “G”, no-go trials with a “N”. Longer inter-trial intervals indicate incorrect responses.

E - Raster plots of lick times showing the last 10 go (left) and no-go trials (right) of representative experiments during the first session of the 500 ms object localization task. The two top panels show raster plots for a single individual per genotype. The bottom panel shows the histograms of the relative timing of the licks over all trials averaged for all performers. The green area (850 ms) refers to the interval during which the stimulation bar moved vertically, either into (go trials) or in front of (no-go trials) the whisker field. The grey area indicates the response window (500 ms).

More WT than L7-PP2B mice learned the localization task

Contrary to the association phase, which could be mastered by all mice, the object localization task with a 2000 ms RW was not learned by all mice. Of the 16 WT mice, 14 learned this object localization task in that they reached a success rate of more than 80% correct trials.
during two consecutive sessions within 35 daily sessions. Significantly less L7-PP2B mice were able to learn this task: only 6 out of 14 mice succeeded (87.5% vs. 42.9%; \( p = 0.019 \); Fisher’s exact test) (left panels in Fig. 5A-B). The mice that did not manage to learn the object localization task with a RW of 2000 ms were considered as non-performers and not tested any further.

**WTs learned the localization task faster than L7-PP2B mice**

WT mice were faster learners than L7-PP2B mice. For example, the fastest WT mouse took four sessions to master the 2000 ms localization task, whereas the fastest L7-PP2B mouse needed eleven sessions. The complete task including both the 2000 ms and 500 ms localization tasks was learned significantly faster by WTs than by L7-PP2B performers (genotype: WT (\( n = 14 \)) 17.4 ± 8.7 vs. L7-PP2B (\( n = 6 \)) 24.8 ± 7.7 sessions; mean ± SD; \( F_{1,54} = 4.395; p = 0.041 \); two-way ANOVA) (Fig. 5A-B). With regard to the 2000 ms object localization task only, it took the L7-PP2B mice longer to learn the task than the WT mice, but this difference did not reach statistical significance (WT: 11.6 ± 7.0 versus L7-PP2B: 18.5 ± 6.9 sessions; mean ± SD; \( p = 0.090 \) (not significant: \( a_{corr} = 0.017 \) (see Methods)); Mann-Whitney test) (Fig. 5A-B, D). The lower learning efficiency of the L7-PP2B mice was also reflected in the slower increase of correct responses during the 2000 ms task (genotype: \( F_{1,27} = 5.098; p = 0.032 \); repeated measures ANOVA) (Fig. 5E).

**WTs learned to fine-tune the timing of their lick responses better than L7-PP2B mice**

When comparing the number of licks during the last (100 ms) bin before the RW and the first bin within the RW we found an interesting difference in the precise lick timing between WT and L7-PP2B mice. At the end of the 2000 ms object localization task, the number of licks was equally distributed between just before and just after the onset of the RW (WT: ratio = 1.01 ± 0.04; L7-PP2B: 1.04 ± 0.09; \( p = 0.758 \)). However, in mice that were trained to time their licks accurately, so at the end of the 500 ms RW phase, WT mice showed an increase in licking just at the start of the RW, contrary to L7-PP2B mice (WT: ratio = 1.13 ± 0.07; L7-PP2B: 0.91 ± 0.05; \( p = 0.019 \)) (Fig. 5C). Likewise, when we reduced the RW from 2000 ms to 500 ms, the L7-PP2B mice required significantly more sessions than their WT littermates (WT: 2.4 ± 0.7 vs. L7-PP2B: 3.8 ± 1.3 sessions; \( p = 0.010 \) (significant: \( a_{corr} = 0.017 \); Mann-Whitney test) (Fig. 5A-B). Thus, while the L7-PP2B mice in general had more difficulties learning the object localization task, the difference with WT mice was especially clear when fast response timing was required.

Importantly, whisker clipping following training with RWs of 500 ms significantly affected the performance during the object localization task (\( n = 10 \) mice; \( p < 0.001 \); paired \( t \) test) (right panel in Fig. 5E) confirming that mice use their whiskers to detect the stimulus bar and respond accordingly. Since not all sessions had an equal number of trials, we also compared the number of trials per individual required for the 2000 and 500 ms task. This confirmed that WT mice were in general faster in learning than L7-PP2B mice (Fig. 5F).
Moreover, together with the more accurate timing of the licks in WT mice (Fig. 5C), these data indicated that the differences among WT and L7-PP2B mice are more prominent with shorter RWs.

Figure 5 - Absence of PP2B in cerebellar Purkinje cells impairs learning of a whisker-based object localization task

A - Summed learning curves of WT mice during the 2000 ms (left panel) and the 500 ms (right panel) object localization task across consecutive sessions (x-axis). The number of trials per session was normalized to 100%. Upon reaching a success rate of ≥ 80% during two consecutive sessions mice continued to the next phase. Performers and non-performers are indicated in blue and grey, respectively. B - Idem for L7-PP2B mice (indicated in red). Insets: Averaged number of sessions to complete the 2000 ms (left) and 500 ms (right) object localization task; * p < 0.017 (t test). C - The fine timing of the lick responses at the end of the 100 ms period preceding the RW and at the first 100 ms period of the RW suggests a cerebellar role in the timing of the decision process to lick. The ratio between the number of licks during the first 100 ms after the onset of the RW and the preceding 100 ms equals 1 during the last session of the 2000 ms object localization task (left), but is increased in trained WT mice (but not in L7-PP2B mice) during the last 500 ms object localization task (right); * p < 0.02. D - Cumulative histograms of the percentage of mice that reached criterion showing that more WT mice were able to learn the object localization task than L7-PP2B and that WT performers were faster than L7-PP2B performers. E - The fraction of correct trials over the sessions. Dark lines show the averages and the shaded areas cover the average ± SEM. For control, we clipped the whiskers of 10 mice (8 WT + 2 L7-PP2B mice) following successful completion of the 500 ms object localization task. Their performance level during the subsequent session (black open symbol) was comparable to that of naive mice and much lower than that during the last session with intact whiskers (dark red closed symbol); * p < 0.001 (paired t test). F - The average numbers of trials the L7-PP2B mice needed to learn the 500 ms and 2000 ms object localization tasks were significantly greater than those in WTs (p < 0.05).
**WT mice show a better learning trajectory than L7-PP2B mice**

Since we found that the WT and L7-PP2B mice differed in their learning skills during the object localization task, we further investigated the relative contributions of their licks during the go trials and the withholding of their licking during the no-go trials to the overall learning process. To this end we plotted the “false alarm” rates (i.e., licking during no-go trials) vs. the “hit” rates (i.e., licking during go trials) for each session of the object localization task. Separate plots of the learning trajectories were constructed in receiver operating characteristic (ROC) space (Fig. 6A). As mentioned before, the mice were trained during the preceding association phase to lick during all trials. Consequently, during the first object localization session (with a 2000 ms RW) they licked very often irrespective of the trial type. As a result, they performed close to guess rate.

During the subsequent sessions, the WT performers markedly and consistently increased accuracy, moving almost along a straight line towards our defined criteria: the green area in Fig. 6A. They continued to lick during the go trials, but decreased their licking during no-go trials. Thus, they maintained a high level of sensitivity to go trials, but specifically reduced their response to no-go trials. After four sessions, the fastest learning WT mice reached criterion (see Fig. 5A). Instead, the L7-PP2B mice reduced their licking during the first four sessions in a random fashion: they stayed close to guess rate. The difference between the WT and L7-PP2B mice during these first four sessions is striking: the WT mice keep licking at the same rate during the go trials, but reduce their licking during the no-go trials. In contrast, the L7-PP2B mice do not initially discriminate between go and no-go trials.

In the subsequent sessions also the six L7-PP2B performers increased their successful licks, but their learning trajectories remained more noisy than those of the WT performers. This difference in learning trajectories is particularly evident when comparing the deviations from the linear regression between the WT and L7-PP2B performers (Fig. 6A inset). The differences in learning strategies are characterized by the chances in the sensitivity index (d’) (WT vs. L7-PP2B: \( p = 0.001 \)) (Fig. 6B). These curves illustrate the superior ability of WT mice in comparison to L7-PP2B mutants to discriminate between go and no-go trials and act accordingly. When the same analysis was done for the 500 ms localization task, which was rapidly learned, the averages of the WT and L7-PP2B performers were already mostly in the “green area”, indicating good performance, from the first session onwards (Fig. 6C). Still, here too, the L7-PP2B performers showed a clear drop in performance during the second session of the 500 ms task.
Figure 6 - WT mice have more efficient learning trajectories than L7-PP2B mice

A - Average false alarm rates (licking during the response window of no-go trials) versus average hit rates (licking during the response window of go trials) in receiver operating characteristic (ROC) space during the 2000 ms object localization task. Perfect classification of both the go trials and the no-go trials would be 0% false alarms and 100% hits. Successful trials (≥ 80% correct) can be found in the green area. Plotted are the averages of all WT (blue) and L7-PP2B (red) performers for 28 sessions (session no. indicated on each symbol), which was the maximum number of sessions required to master the 2000 ms object localization task. Linear regression lines are indicated. Note that the WT mice decrease the number of false alarms from the beginning on, while the L7-PP2B mice first generally reduce the licking responses, irrespective of the trial type. Inset: Summed least squared differences between the first 28 sessions and the linear regression lines for WT and L7-PP2B performers during the 2000 ms object localization task. B - The sensitivity index (d') of all animals (0 = chance performance; 1.68 = 80% correct trials). C - The same plot as A, but for the 500 ms object localization task. Note that the WT mice are in the green area from the start on, while the L7-PP2B mice initially show a decreased performance relative to the previous phases of the object localization tasks.
Discussion

L7-PP2B mice show impaired cerebellar plasticity (Schonewille et al., 2010). Here we demonstrate that L7-PP2B mice clearly have problems in learning a whisker-dependent object localization task. Not only are fewer L7-PP2B mice able to learn the object localization task, they also need a longer time to do so and the fine-tuning of the precise timing of their learned responses is deficient. Moreover, the learning trajectories of L7-PP2B mice are much more erratic than those of WT mice: the L7-PP2B mice show a decreased ability to keep responding reliably during go trials while at the same time stopping to respond during no-go trials. The differences between WT and L7-PP2B mice were especially clear under strict timing requirements. These differences in learning performance could not be explained by motor or sensory problems, since neither the licking pattern nor the association of sensory stimulation and lick responses were significantly altered in L7-PP2B mice. Hence, we conclude that cerebellar plasticity is involved in learning the whisker-dependent object localization task.

Can the observed learning deficits be explained by sensory and/or motor problems?

It has previously been reported that L7-PP2B mice do not show overt signs of motor ataxia (Schonewille et al., 2010). However, no special attention to licking or whisking behavior was given at that time. Licking and whisking are both rhythmic behaviors under control of a brainstem pattern generator (Moore et al., 2013). However, both licking (Bryant et al., 2010) and whisking (Lang et al., 2006; Bosman et al., 2010) have neural correlates in the cerebellum. For these reasons we first investigated the licking behavior of L7-PP2B mice. We analyzed licking patterns in freely moving and head-restrained mice. Under all circumstances, the dominant licking behavior was rhythmic licking with a frequency around 8 Hz, which is in line with previous reports (Horowitz et al. 1977, Wiesenfeld et al. 1977; Yamamoto et al. 1982). Nevertheless, rhythmic licking at 8 Hz was reduced during the first session of the association phase when the mice were head-restrained, compared to licking in the home cage. Indeed, lick frequencies can be context-dependent (Weijnen, 1998). Still, all mice apparently got used to licking in the head-restrained situation, since 8 Hz rhythmic licking increased again after the initial stages of habituation and the association task. This phenomenon occurred in both WT and L7-PP2B mice. During the last phase of the object localization task, with a RW of 500 ms, relatively many high-frequency licks were observed. At that stage, the water was available for only 500 ms, and this might in principle have triggered fast licking and thereby evoked additional phenotypes. Yet, WT and L7-PP2B mice showed also in this respect a similar behavior. Thus, although the 8 Hz rhythmic licking was always the most prevalent form of licking, some plasticity in licking behavior took place in both WT and L7-PP2B mice. We conclude that the deficit in learning of the L7-PP2B mice could not be explained by difficulties with licking.

Another possible cause for performance deficits could have been abnormal whisker
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exploration. Mice explore their environment by making large, rhythmic whisker movements. Such exploratory whisking occurs at frequencies between 5 and 15 Hz (Berg and Kleinfeld, 2003; Cao et al., 2012). The absence of rhythmic whisking, or at much lower frequencies, could point to a sensory deficiency. Therefore we recorded free whisking behavior in head-restrained mice and found that both WT and L7-PP2B mice showed bouts of rhythmic whisking at comparable frequencies. During these whisking bouts, the whiskers scanned the whole area within reach of the whiskers. We also tested whether mice from both genotypes actively whisked during the insertion and presence of the stimulus bar, and this was the case in all trials tested. Thus, L7-PP2B mice did not show abnormalities in the timing, frequency or amplitude of whisker movements.

Sensory ability was finally tested with the association task. All mice were able to associate the insertion of a bar into the whisker field with a water reward. The time to learn to associate the stimulus with the correct response was similar for WT and L7-PP2B mice. Hence we conclude that the L7-PP2B mice did not have sensory and/or motor problems that could explain the observed learning deficits during the subsequent object localization task.

Response timing

The cerebellum is required for both predictive and reactive timing of responses (e.g. De Zeeuw et al., 2011). This has for example emerged from eyeblink conditioning tests. A series of cerebellar mutants, including L7-PP2B mice, have problems to learn the accurate timing of eyelid responses (Koekkoek et al., 2003; Schonewille et al., 2010). Although our learning paradigm did not require very strict timing, we still observed a striking difference in the lick timing at the onset of the RW - so exactly at the time the water became available. Trained WT mice showed an increase in licking at this moment under the strict temporal constraints of the 500 ms RW, whereas L7-PP2B mice did not do this (Fig. 5C). This may indicate that - apart from the general learning deficits - also a deficit in learning-dependent timing is present in L7-PP2B mice.

Different learning strategies

Comparing the licks during the RW of go and no-go trials yielded interesting insights in the learning strategies. WT mice, after completion of the association task, initially licked during most trials, irrespective of whether they were subjected to go or no-go trials, creating a response bias. Rapidly, however, they reduced their licking during no-go trials, creating a response bias, while continuing to lick during go trials. Thus, in ROC space (Fig. 6A), they moved almost along a horizontal line towards the goal area. L7-PP2B mice also started with licking during most trials, but they initially seemed to fail to make a distinction between go and no-go trials: they reduced their lick responses equally during both types of trials. Only later on, they started to make better discriminations, but their improvement over trials was significantly less than in WT mice.
Cerebellar plasticity and cerebello-cerebral connections

L7-PP2B mice are deficient in the induction of intrinsic plasticity of their cerebellar Purkinje cells and lack expression of LTP at their parallel fiber-to-Purkinje cell synapses. However, LTD of their parallel fiber-to-Purkinje cell synapses is intact (Schonewille et al., 2010). The learning deficits observed during eyeblink conditioning and adaptation of the vestibulo-ocular reflex (Schonewille et al., 2010) challenge the theory that parallel fiber LTD is the main mechanism underlying cerebellar learning (Ito, 2001). We show here that L7-PP2B mice also have problems to learn a whisker-dependent object localization task.

Such a learning task is not generally considered to be a cerebellar learning task. Indeed, it has been shown in a series of experiments to require plasticity in the cerebral cortex (O’Connor et al., 2010a; Huber et al., 2012; Petreanu et al., 2012; Xu et al., 2012). In the present study we show, using a Purkinje cell-specific mutant mouse line, that cerebellar plasticity may also contribute to learning a whisker-dependent object localization task in which mice have to respond within a certain response window. In this respect the current localization task diverges from for example the standard Morris water maze test or other global tasks such as the open field task, fear conditioning and social interaction tasks, during which the temporal constraints are less tight and the L7-PP2B mice do not show learning deficits (Galliano et al., 2013). Cerebellar activity can amongst others prime M1 plasticity (Popa et al., 2013b) and promote gamma band coherence between the barrel cortex and the whisker motor cortex (Popa et al., 2013a). These findings highlight a potential role of the cerebellum in facilitating neocortical learning when temporal constraints are relevant. We found here that a reduced subset of L7-PP2B mice was able to learn the whisker-based object localization task and that the L7-PP2B performers were considerably less efficient in learning than their WT littermates. Thus our results suggest that intrinsic plasticity of Purkinje cells and/or parallel fiber LTP can facilitate neocortical learning when timing is critical.
References


CHAPTER IV
General Discussion and Future Aims
4.1 General Discussion and Future Aims

4.1.1 General Discussion

In chapter 2.1 we aimed to investigate two questions, does the hippocampus mainly support spatial information processing as some had suggested [1, 2] and is recognition memory supported by two independent types of retrieval? We found further evidence for the hippocampus supporting non-spatial memory and that the hippocampus supports recollection for items in context and not familiarity as a vague sense of knowing. Studies have found since then, the role of the medial prefrontal cortex in supporting recollection but not familiarity, while amygdala lesions selectively impair familiarity but not recollection [3, 4]. In chapter 2.2 we found that dorsal hippocampus rarely uses trajectory coding during a tactile-visual conditional discrimination task alone, but often uses trajectory coding in a continuous spatial alternation task. However later reports indicated that experience with multiple tasks reduces the likelihood of neurons representing distinct trajectories and that remapping occurs between a delayed alternation task and the same conditional discrimination task as in chapter 2.2. Rate remapping does occur during continuous alternation and conditional discrimination, possibly indicating the role of memory load on hippocampal remapping [5]. Finally, time cells in the same region were discovered in which encoding occurs during empty temporal gaps in a sequence and that these cells “retime” when temporal parameters are adjusted to a sequence, similar to spatial remapping indicating that temporally organized memories may be represented in hippocampus similarly to how place cells represent locations [6]. Therefore, the difference between trajectory dependent coding in continuous alternation and its lack in our tactile-visual conditional discrimination task, may hint at the importance of temporal components in a task but may also be related to the proximodistal location of the cells [7]. Whereas a discrete cue allows the rat to make the decision at the choice point, delayed alternation and continuous alternation requires the animal to know where it was last in order to be successful. Perhaps, most importantly, it was found that the same conditional discrimination task required the striatum while the delayed alternation task required hippocampus [8]. However, as mentioned, trajectory dependent coding was seen in a continuous alternation task which is also not hippocampal dependent. Therefore it is likely that trajectory dependent coding may be due to extrahippocampal inputs [9] located in the entorhinal cortex.

Challenges in pinpointing functional correlates in integrative brain regions are exemplified through the history of discoveries of temporal lobe function. The discovery of grid cells was preceded by the discovery of place cells and head-direction cells. Post-subiculum and medial entorhinal cortex input representing head-direction cells and grid cells gives the animal egocentric and allocentric input important for the animal to locate itself in space. Since then head direction cells have also been found in anterior thalamus, lateral mammillary nuclei, medial entorhinal cortex and other areas [10-13].
So, head-direction cells respond to head direction by increasing or decreasing firing dependent upon the cell and the egocentric position of the head. Grid cells also employ a temporal code by increasing firing in specific locations of the animal’s environment. In turn, place cells also use a temporal code. That is, a neighboring cell in the hippocampus does not represent a neighboring place in the animal’s environment. Also, instead of a continuous representation of space along the entire entorhinal border using a spatial code, it seems the lateral entorhinal cortex codes better for the identification of objects [14], medial entorhinal cortex codes better for space, and theta rhythms may enhance spatial firing accuracy upstream of the entorhinal - hippocampal pathway [7]. The cerebellum utilizes its architecture and requires spatiotemporal patterns for functional processing [15]. It contributes to vestibular processing and it is hypothesized that parallel fibers specifically assist in orienting the body [16].

Although the whisker system is an ideal model for investigating physiological correlates of functions such as learning, other behavioral paradigms have dominated cerebellar investigations. We aimed to gain a better understanding of basic encoding characteristics of sensory input to the cerebellum. Chapter 3.1 describes differential Purkinje cell complex spike and simple spike responses to whisker stimulation and spiking ensemble orientations related to cerebellar anatomy. Chapter 3.2 aims to continue these basic questions. We asked whether sensory input will have differential Purkinje cell post complex spike - simple spike responses during whisker stimulation in the absence of molecular layer interneuron input. We found that whisker stimulation decreases the proportion of cells with post-complex spike - simple spike facilitation in normal animals and that this post-complex spike activity is related to firing frequency. Mutants lacking molecular layer inhibitory input did not show these changes indicating a possible mechanistic role for post-complex-spike simple spike activity in synaptic plasticity related to Purkinje cell inhibitory input.

In chapter 3.3 we asked whether the cerebellum and specifically cerebellar plasticity might contribute to learning a cerebral cortex dependent, whisker-based object localization task. In this task we trained L7-PP2B mice which exhibit impaired intrinsic Purkinje cell plasticity and long term potentiation at their parallel fiber to Purkinje cell synapses, and littermate controls. These mice were first trained to lick within two seconds upon whisker stimulation (go trials only) via vertical rise of a metal pole. Upon reaching two sessions of successful performance of this task the mice then received no-go trials as well as go trials while the response window remained the same. Now the mice had to localize the object in egocentric space using its whiskers, for optimal reward. If mice reached successful performance of this task the response window was decreased to one second and finally 500ms. We found that less mutants were able to learn the go no-go task with a 2 second response window and that those that did, required more trials to obtain criteria for successful performance. These data indicate a relevant role of cerebello-cortical interactions in a whisker based associative learning task.
**Strengths and weaknesses**

There are strengths and many weaknesses in *Chapter 3.2*. One of the strengths in using a multielectrode array is to gain an understanding of spatial firing patterns intrinsic to the unique cerebellar cytoarchitecture. We hypothesized that simultaneously recorded cells in local cerebellar zones will have similar firing properties. Although methods such as 2-photon calcium imaging has better spatial resolution, we hope to gain an understanding from this viewpoint from our data in both simple spike and complex spike firing. This analysis is not included in this thesis. Another strength of methods used in this chapter is in utilizing knockout mice to gain an understanding of the role of inhibitory input to the Purkinje cell during sensory (whisker) stimulation. However in order to say something concrete about how simple spike firing is affected during such input, simple spike responses triggered on the actual whisker stimulation is essential. This data is not included in this thesis. Furthermore, some complex spikes in the raw data may be triggered by the air puff while others are intrinsic to the firing of the cell itself. Post-complex spike - simple spike responses should be separated based on these parameters for further understanding of the post-complex spike simple spike responses. We hypothesized that a reduction in inhibitory input to the Purkinje cell would increase incidence of facilitation overall and decrease incidence of suppression in the L7γ2 mutants, but we actually saw an average increase in suppression (data not included in this thesis) presumably due to compensatory mechanisms. However this chapter was laid out as a framework for understanding such complexities. This chapter attempts not to confuse “differences” with “changes” in firing patterns, as cells in each group (WT stimulation and non-stimulation, L7γ2 stimulation and non-stimulation cells) are independent and not continuous recordings. Therefore the data should not be interpreted as such. However, further analysis to understand how muted feed-forward inhibitory input to the Purkinje cell affects changes within cells during such responses is under way. Finally, one of the goals of this research is to parse firing patterns associated with sensory response and firing patterns associated with active whisker movements. In order to do this a rigorous analysis of whisker movements is necessary. These data are not included in this thesis as this manuscript is currently “in preparation”.

**Similarities and differences between hippocampus and cerebellum**

Recently it has been argued that the cerebellum might participate in the construction or maintenance of hippocampal spatial representation maps. There is evidence in monkey that cerebellar vermis transforms head centered vestibular afferent information into self motion and spatial orientation signals [17, 18]. Purkinje cell complex spike activity has also been shown to distinguish optic flow patterns important for locomotor activities in pigeons [19]. Furthermore, mice lacking cerebellar LTD presented impaired hippocampal place cell firing properties [20]. These data are interesting and perhaps not surprising considering the role of head-direction cells, which strongly depend on vestibular input, in maintaining place cell representations in hippocampus.
Until now, the striatum and the basal ganglia have been implicated, more than the cerebellum, for more cognitive types of procedural learning in conditional discrimination tasks [21]. However, we show in chapter 3.3 that the cerebellum plays a role in learning a task that requires cortical recruitment. Although it is well established that the cerebellum assists in motor memory, it may share resources with declarative memory systems pointing to shared resources but also distinct functions [22-24].

For example, tasks such as saccade adaptation and reaching adaptation are learned fast but also decay quickly whereas the slow component of motor memory is also more immune to such decay. Similarly, rapid learning and fast forgetting also occurs in declarative memory systems and therefore it is no surprise that the fast component of procedural memory interferes with declarative memory whereas the slow component of, for example, a reaching task does not interfere. It is hypothesized that the shared resource might be working memory [25].

In hippocampus theta rhythms are large amplitude, slow (4-12Hz) and highly regular signals that play a role in spatial and episodic memory processing. Theta occurs in many hippocampal efferent and afferent structures synchronizing widespread networks. During active exploration both whisking and sniffing correlate with the theta cycle [26]. Gamma oscillations in hippocampus are fast (25-100 Hz) and occur in bursts riding on top of the theta cycle. These signals may selectively group neural ensembles for processing and are well suited for operations beyond the range of conscious perception [27].

Interestingly, there is evidence that gamma oscillations coordinate the transfer of information from medial-entorhinal cortex, CA1, and CA3, where medial entorhinal cortex is more effective at transmitting information to CA1 (region important for encoding) during fast gamma, and CA1 is more effective at transmitting information to CA3 (region for retrieval) during slow gamma. Therefore there is evidence that gamma oscillations may orchestrate encoding and retrieval processes in hippocampus [28, 29].

Furthermore, theta-frequency bursts at the granule cell level of cerebellum have been shown to facilitate potentiation at the Purkinje cell [30]. Mutant mice (as shown in this thesis) lacking potentiation at the parallel fiber to Purkinje cell synapse show severe learning deficits in a whisker based object localization task that requires S1 for performance. Inhibiting cerebellar output disrupts M1/S1 gamma coherence [31]. Therefore oscillations, especially theta and gamma oscillations, may play a large role in orchestrating function among and within brain regions.
4.1.2 Future Aims

Long term future aim: To dissociate the contributions of cerebellum and hippocampus to the brain processes involved in spatial and tactile discrimination learning and memory.

1. **Do mice use different modes of whisking during a spatial vs. tactile discrimination task?**

Rats adapt their whisking mode depending on the behavioral context and the information required to perform a task [32]. However whether mice can also adapt whisking related to context has not yet been answered. We show in chapter 3.1 that climbing fiber input is sensitive to amplitude, speed and direction of passive whisker movement in anesthetized mice. We also show in chapter 3.1 that parallel fiber input is sensitive to passive movement of multiple whiskers in anesthetized mice. Furthermore, we show in chapter 3.2 that awake mice exhibit a larger ratio of cells with facilitation during whisker stimulation using an airpuff and that these dynamic changes are less robust in mutants with disrupted molecular layer interneuron inhibitory input. Finally, in chapter 3.3 we show the cerebellar plasticity facilitates learning of a whisker based spatial discrimination task. Although many investigators use passive stimulation of the whiskers to investigate neural properties of sensory input, the cerebellum partakes in coordinating both sensory input and motor output. Therefore understanding differential whisker kinematics utilized during an associative conditioning task will lay the ground work for cerebellar electrophysiological investigations of mechanisms such as feed forward inhibitory input and parallel fiber - Purkinje cell plasticity known to be important for cerebellar learning.

2. **How do Purkinje cells encode whisker stimuli (sensory) and/or whisker movements (motor)?**

Future aim 2 is to characterize normal and irregular Purkinje single cell firing in relation to whisker stimulation and spontaneous whisking. Without an understanding of characteristic Purkinje cell firing patterns during different behavioral states, further investigation of cerebellar learning during more complicated whisker related responses will be challenging. However this analysis should be possible with data already obtained in our group.

The functional role of climbing fibers and parallel fibers in processing whisker input is also not yet well understood in the awake, behaving animal. Since the cerebellum receives convergent information from cortical and subcortical structures we will correlate cell ensembles to whisker sensory stimulation and whisker motor output (See chapter 3.2). Areas crus 1 and crus 2 of the cerebellum respond with different firing properties to whisker input. However little is known about how or if these regions contribute to normal whisking [31].

Although the cerebellum has been classified as responsible for only procedural memory that involves the modification of motor responses, and not conditional discrimination, task
switching, or reversal learning [33, 34] our data does show severe impairment in mutants lacking cerebellar plasticity in switching from a detection task to a discrimination task where perseverative licking seems to influence performance. This viewpoint supports evidence that cerebellar disfunction plays a role in perseveration [35]; [36]; [37], that is also exhibited in autism spectrum disorder [38]), a disorder where the cerebellum has been implicated.

This is most readily illustrated in the learning curves plotted as a receiver-operating characteristic in the first several sessions of the detection task in chapter 3.3 figure 6. It can be seen that the mutants show a difficulty in retaining hit rates while also reducing false alarms, while wild-type mice do not. Furthermore, it has been shown that parallel fiber to Purkinje cell LTP induction is crucial for VOR adaptation in mice and cerebellar activation in humans may play a role in the automated preparation when multiple responses are necessary [24, 39]. Therefore deficits in cerebellar plasticity, may cause deficits in licking adaptation.

It has also been shown that temporary inactivation of contralateral barrel cortex during whisker based object localization reduces performance levels to chance [40]. Therefore, it is possible that a reduction in cerebellar plasticity may contribute to deficits in S1-M1 coherence thereby causing deficits in orchestrating whisking and licking behavior contiguity. This point is further reinforced by evidence showing strengthened lick related population coding in M1 layer 2/3 neurons [41].

Finally, a more complicated alternative and less well established hypothesis to the findings in chapter 3.3 may be due to cerebellar contributions to spatial event processing evidenced in a right-left discrimination task for rodents [42]. If there is a cerebellar role in functions of spatial representation and integration of multi-source self motion information, one might expect cerebellar plasticity deficits to affect a whisker based object localization task [20].

If future aims 1 and 2 are successful we should have an understanding of the role of Purkinje cell climbing fiber and mossy-fiber input during free and exploratory whisking. Some working hypotheses from our group is that the inferior olive may reset the phase of the whisking cycle or that it may play a role in either the adaptation of licking or whisking behavior, or both. There is also evidence of a functional segregation for whisking at the level of the thalamus. [43](see figure 4.1). Whether this functional segregation is unitized physiologically in the cerebellum remains to be seen.

In regard to the cerebellum, we and others show that Purkinje cells in areas such as crus I, crus II, paramedian lobule, simplex and vermis respond to whisker stimulation from airpuffs but we do not know if there is a response in these regions during active whisking in tasks such as that in chapter 3.3. We and others do see modulation of simple spike firing during licking behavior as well, but we do not know if there is other context and task dependent
information in the cerebellar firing codes, either in single cells, or cell ensembles. Perhaps more eloquently stated, “If, for example, a rat could be trained to discriminate between two textures that evoke equivalent firing rates, but distinctive firing patterns and the spike pattern present in single trials could predict behavior, this would constitute strong evidence that spike patterns encode texture” [44].

3. What are the contributions of cerebellum and hippocampus during spatial and tactile discrimination tasks?

By using stimuli in the same parametric space and time, but with different required contextual responses to those stimuli, an information bottleneck can be created when recording from the same cells. We can train an animal by rewarding it for licking when a bar is in the anterior (go) position but not the posterior position (no-go) for a first set of trials and then train the animal on a second block of trials where the animal is rewarded for the smooth surface (go; but either in the anterior or posterior position) but not the rough surface (no-go) trials (see figure 4.1). The coding properties then, including spatial and temporal patterns in cerebellum can be defined at a specific time point in well-defined space, generating differential task specific “meaningful” responses.

Next, varying discriminability thresholds we can thereby parametrically challenge the cerebellum’s abilities for sensorimotor integration and or recalibration of sensory predictions about external stimuli. Training mice to do spatial discrimination with varying airflow perturbations/strengths will build upon the work we have already done using air puff stimulation and object localization, and falls in line with what we already know the cerebellum can do.

How does the animal adapt whisking behavior to compensate for perturbations in the normal whisking cycle? If complex spikes reset the whisking phase, but also respond to air puff stimuli, how quickly can this adaptation take place, and how will simple spike and complex spike ensemble firing change and/or affect each other? When multiple behavioral states require adaptation (whisking and licking), what state takes precedence (if any) if the inferior olive is signaling such adaptation? If the cerebellum dictates M1-S1 coherence, what is the psychometric discriminability threshold and how does it manifest neurometric thresholds in each respective brain region and as an olivo-cerebello-cortical system?

Final Statement

In chapter 2.1 it is not JUST a sensory discrimination that is processed in the hippocampus, it is a unitized event of an odor-medium pair that must be discriminated. In chapter 2.2 we were not searching for cells that encode visual or tactile features in hippocampus, but cells that code for a whole context that will predict what decision the rat should make. In chapter 3.1 we show that cerebellar Purkinje cells “encode” the stimulus, but in order to know why they
show differential firing properties for meaningful output. In chapter 3.3 we chose a task that requires sensory representations at the level of the sensory cortex for successful performance. However, cerebellar plasticity was not required for successful performance, 6 out of 14 mutant mice completed the whole task. One would expect that if sensory representations were coded and necessary in cerebellum those mutant animals would not have completed the task.

But this is precisely why when investigating brain regions that integrate so much information, it is valuable to peer into the brain from the outside in, in addition to the inside out. Therefore, maybe this thesis is not just about neural representations in sensory discrimination, but the utility of manipulating the dependent variable of behavior from the perspective of outer psychophysics, to obtain neural representations of the function of the brain region of interest. This concept is vividly evident in the history of discoveries made in hippocampal and parahippocampal function. Where there was once a place cell in hippocampus, there also came context dependent cells, trajectory dependent cells, and time cells. In parallel were discoveries of head direction cells and grid cells, but all only after that first discovery did the rest follow, and as the rest followed so does a better understanding of how multiple brain regions represent meaning in episodic memory. The role of cerebellum in procedural memory may also follow suit. However, when place cells were discovered, it was evident through temporal coding, higher firing of a cell in one place. If the cerebellum uses a spatiotemporal firing code for multiple functions, the complexity of that code perhaps, exponentially multiplies. Therefore the behavioral constructs for investigating cerebellar physiology and function must be diverse but also controlled.
Figure 4.1: A simplified connectivity map of the whisker system. Somatotopic representations of the whisker system occur from whisker to cortex. The cerebellar cortex does represent whisker input but with a fractured somatotopy. Sensory cortex (SI), secondary somatosensory cortex (SII), entorhinal (ERH) and frontal cortex (FC). Motor connections are indicated in gray.
References

APPENDIX

Summary
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Acknowledgements
5.1 Summary

Memory can be divided into two broad categories; declarative and nondeclarative memory. This thesis aims to uncover how brain structures and their physiological representations of sensory stimuli are encoded, unitized and distinguished with the long term goal of investigating mechanistic similarities in regions necessary for learning and memory.

Learning is the acquisition of new knowledge while memory is the retention of learned information. Declarative memory is the memory of facts and events while non-declarative memory is the memory for functions such as basic motor learning, skills and habits, associative and non-associative learning.

The hippocampus (among other regions) is known to support declarative memory while the cerebellum (among other regions) supports motor learning, basic associative learning, and acquisition of skills and habits. In order to understand behavioral and neural coding properties in the hippocampus and cerebellum for the above described functions, experiments were conducted in awake and anesthetized animals. Behavioral experiments used lesion methods in rat or specific mutant mice to learn about the contribution of that particular region to the stimulus or task. All behavioral tasks used some form of conditional discrimination. Electrophysiology experiments consisted of extracellular multiple unit recordings to discover physiological encoding properties for that particular stimulus or function.

Declarative memory for facts and events has also been termed semantic and episodic memory respectively. It has been hypothesized that the hippocampus is relational and conjunctive in that it relates “items” in “context” where recollection recalls an entire event and familiarity only occurs when parts of an event are recalled, such as the item only or context only. We aimed to investigate if familiarity and recollection are mediated by distinct brain regions. Chapter 2.1 utilizes the rodent’s keen sense of olfaction and somatosensation showing that the hippocampus is necessary for recollection but not familiarity of previously learned, odor-medium paired associations. We also added further evidence that the hippocampus is not only involved in spatial memory since all stimuli were delivered in the same place yet deficits were still apparent after lesioning the hippocampus.

Although we showed that the hippocampus does not only contribute to spatial memory, we do know without a doubt it does mediate spatial memory. In chapter 2.2 we sought to investigate whether dorsal hippocampal CA1 pyramidal cells hold more information than just space. There is evidence that hippocampal cells sometimes fire differentially in overlapping paths predicting where the animal is coming from or going to in a given trajectory. We found further evidence that while trajectory dependent firing in CA1 of hippocampus may occur in both hippocampal and non-hippocampal dependent tasks, trajectory dependent coding may require working memory. Therefore discrete cues during expert performance do not facilitate
such characteristic coding properties. Hence, the hippocampus is conjunctive and relational. It supports declarative memory that can be spatial or non-spatial and does not necessarily exhibit trajectory dependent firing.

Just as investigations of spatial memory in the hippocampus dominated after the discovery of place cells, research on the cerebellum has often focused on a small handful of behavioral paradigms. However the whisker system is an ideal model for investigating physiological correlates of functions such as learning, and is gaining traction as useful model. We aimed to gain a better understanding of basic encoding characteristics of sensory input to the cerebellum. The two major excitatory inputs to the cerebellar cortex include the climbing fibers and the parallel fiber-mossy fiber pathway. Chapter 3.1 describes differential purkinje cell responses to whisker stimulation and spiking ensemble orientations related to these two input pathways and found that climbing fiber input encodes for single whisker input, speed and whisker direction while the mossy fiber-parallel fiber pathway encodes for multiple whisker input. Chapter 3.2 aims to continue these basic questions. We asked whether sensory input will have specific interactions between the two excitatory pathways during whisker silent periods and stimulation in the absence of inhibitory input to the cerebellar cortex. We found that whisker stimulation decreases the proportion of cells with post-complex spike - simple spike facilitation in normal animals and that this post-complex spike activity is related to firing frequency. Mutants lacking molecular layer inhibitory input did not show these changes indicating a possible mechanistic role for post-complex-spike simple spike activity in synaptic plasticity related to cerebellar cortex inhibitory input.

In chapter 3.3 we asked whether the cerebellum and specifically cerebellar plasticity might contribute to learning a cerebral cortex dependent, whisker-based object localization task. In this task we trained mutant mice which exhibit impaired cerebellar plasticity and their littermate controls. These mice were trained to lick upon whisker stimulation (go trials only) via vertical rise of a metal pole. Upon reaching successful performance of this task the mice then received no-go trials as well as go trials while the response window remained the same. If mice reached successful performance of this task the response window was decreased to test the role of cerebellar plasticity in timing. We found that less mutants were able to learn the go, no-go task with a long response window and that those that did, required more trials to obtain criteria for successful performance with a short response window. These data indicate a relevant role of cerebello-cortical interactions in a complex cognitive whisker based associative learning task.
5.2 Samenvatting

Het geheugen kan verdeeld worden in twee grote categorieën; decleratief en niet-decleratief geheugen. Deze thesis heeft als doel om te ontdekken hoe hersenstructuren en hun fysiologische representatie van sensorische stimuli worden gecodeerd, verenigd en onderscheiden. Op de lange termijn is het streven om meer inzicht te vergaren in mechanische overeenkomsten in gebieden die nodig zijn bij leren en geheugen.

Leren is het vergaren van nieuwe kennis, terwijl geheugen het ophalen van geleerde informatie. Het decleratieve geheugen omvat geheugen met betrekking op feiten en gebeurtenissen, terwijl het niet-decleratieve geheugen functies als basis motorisch leergedrag, vaardigheden en gewoontes en associatief en niet-associatief leren omvat.

Een van de gebieden die bekend is betrokken te zijn bij het decleratief geheugen is de hippocampus. Het cerebellum daarentegen is een van de gebieden die bekend is betrokken te zijn bij motorisch leergedrag, basis associatief leren en het verkrijgen van vaardigheden en gewoontes. Om te begrijpen hoe de bovenstaande functies in relatie staan met gedrag en neurale codering in de hippocampus en het cerebellum zijn er experimenten uitgevoerd in wakkere en verdoofde muizen. De gedragsexperimenten maakten gebruik van laesie methodes in ratten of specifieke muismutanten, waarmee de bijdrage van een specifiek gebied bij een stimulus of taak onderzocht werd. Alle gedragstaken maakte gebruik van een soort van conditionele discriminatie. Electrofysiologie experimenten bestonden uit extracellulaire multiple unit opnames om de fysiologische coderingseigenschappen van de specifieke stimulus of functie in kaart te brengen.

Decleratief geheugen voor feiten en gebeurtenissen staat ook wel bekend als respectievelijk semantisch en episodisch geheugen. De hypothese is dat de hippocampus relationeel en verbindend is, omdat het items in een context plaatst. Hierbij haalt recollectie een gehele gebeurtenis op, terwijl bekendheid alleen plaatsvindt als delen van een gebeurtenis worden opgehaald, zoals item of context alleen. We hebben ons gekeken of bekendheid en recollectie van geheugen bepaald worden door aparte hersengebieden. Hoofdstuk 2.1 maakt gebruik van het feit dat knaagdieren een scherp reukvermogen en somatosensorisch vermogen hebben. Hierin wordt aangetoond dat de hippocampus betrokken is bij de recollectie, maar niet bij de bekendheid van de eerder geleerde geur-gemedieerd gepaarde associaties. Tevens hebben we meer bewijs gevonden dat de hippocampus niet alleen betrokken is bij spatieel geheugen, gezien het feit dat alle stimuli geleverd werden op dezelfde plek, maar er waren nog steeds gebreken schijnbaar na het aanbrengen van laesies in de hippocampus.

Ondanks dat we hebben laten zien dat de hippocampus niet alleen een bijdrage levert aan spatieel geheugen, kunnen we met zekerheid stellen dat het spatieel geheugen bepaald. In hoofdstuk 2.2 trachten we te onderzoeken of de CA1 piramidale cellen in de dorsale hippocampus meer informatie bevatten dan alleen ruimte. Er is bewijs dat de hippocampale cellen soms verschillend vuren in paden die kunnen voorspellen of een dier naar een bepaalde
plek toe gaat of van die plek afkomt in een bepaald traject. Tevens hebben we bewijs dat terwijl traject afhankelijk vuren in CA1 van de hippocampus zowel kan voorkomen in hippocampale als niet-hippocampale afhankelijk taken, traject afhankelijke codering wellicht werkgeheugen nodig heeft. Daarom schieten subtiële signalen voor deze karakteristieke coderingseigenschappen tekort. Vandaar dat de hippocampus verbindend en relationeel is. Dit ondersteund het decleratief geheugen dat spatieel en niet-spatieel kan zijn en het is niet nodig bij traject afhankelijk vuren.

Terwijl onderzoekers naar spatieel geheugen in de hippocampus overheerste na de ontdekking van ‘place cells’, focuste onderzoek naar het cerebellum zich vaak op een klein aantal gedragssparadigma’s. Echter is het snorhaarsysteem een ideaal model voor het onderzoeken van fysiologische correlaties bij functies als leren. We streedden naar een beter inzicht in de basis coderingskarakteristieken van sensorische input in het cerebellum. De twee grote excitatoire inputs naar de cerebellaire cortex omvat de klimvezels en het parallel vezel - mosvezel pad. **Hoofdstuk 3.1** beschrijft differentiële purkinje cel reacties door snorhaarstimulatie en groepsgewijs vuren gerelateerd aan die twee inputpaden. Hierbij werd gevonden dat de klimvezelinput codeert voor een enkele snorhaarinput, snelheid en snorhaarrichting. Terwijl het mosvezel - parallel vezel pad codeert voor meerdere snorhaarinputs. **Hoofdstuk 3.2** doelt op het voortzetten van deze basis vragen. Wij stelde hierbij de vraag of sensorische input een specifieke interactie heeft tussen de twee excitatoire paden tijdens snorhaar stille periodes en in afwezigheid van inhibitoire input naar de cerebellaire cortex. We vonden dat snorhaarstimulatie de proportie van cellen met post-complex spike activiteit gerelateerd is aan de vuurfrequentie. Mutanten met een tekort aan inhibitoire input vanuit de moleculaire laag lieten deze veranderingen niet zien. Dit wijst op een mogelijk mechanische rol voor post-complex-spike simpel spike activiteit in synaptische plasticiteit gerelateerd aan inhibitioire input vanuit cerebellaire cortex.

In **hoofdstuk 3.3** stelden we de vraag of het cerebellum en specifiek cerebellaire plasticiteit wellicht bij droeg aan het leren van een cerebrale cortex afhankelijke, snorhaar-gebaseerde object lokalisatie taak. In deze opstelling trainde we mutantmuizen, die een verminderde cerebellaire plasticiteit vertoonden, met hun wild type muizen uit hetzelfde nest. Deze muizen werden getraind om te likken op basis van snorhaarstimulatie (alleen go trials) via het verticaal opstijgen van een metalen paaltje. Bij het bereiken van een succesvolle prestatie van deze taak, werden naast de go-trials, no go-trials aan de taak toegevoegd. De reactietijd blijf hierbij wel hetzelfde. Als muizen een succesvolle prestatie behaalde werd de reactietijd verminderd om te kijken naar de rol van cerebellaire plasticiteit bij timing. Hierbij werd gezien dat mutanten minder goed in staat waren tot het leren van de go no-go taak met een grote reactietijd. Zij die dit wel konden hadden meer trials nodig om de criteria voor een succesvolle prestatie met korte reactietijd te bereiken. Deze resultaten indicieren een relevante rol van cerebellaire-corticale interactie in een complexe cognitieve snorhaar-gebaseerde associatie taak.
5.3 Curriculum vitae

PERSONAL INFORMATION

Name       Cullen B. Owens
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EDUCATION

Graduate

Spring 2010 - Present

Doctoral Program in Neuroscience: Erasmus Medical Center, Rotterdam, The Netherlands

Fall 2008- Spring 2010

Pre-Doctoral Program in Psychology / Behavioral Neuroscience: University of Delaware, Newark, DE

2007-2008

Master of Arts in Psychology: Boston University, Graduate School of Arts and Sciences, Boston, MA

1999-2000

Paralegal Certificate: Northeastern University, Professional and Continuing Education, Boston, MA

Undergraduate

1995 - 1999

Bachelor of Arts in Philosophy: Boston University, College of Arts and Sciences, Boston, MA

RESEARCH EXPERIENCE

Lab Research

Spring 2010 - Present

Doctoral Student: DeZeeuw Laboratory: Erasmus Medical Center, Rotterdam, The Netherlands:
Project involves investigating the functional role of cerebellum as it relates to physiological correlates of whisker kinematics and associative learning in a whisker-based conditional discrimination task. We use in-vivo, single cell, multi-unit extracellular recordings in cerebellum of various mouse mutants during acquisition of whisker movements to ascertain the involvement of the cerebellum during a spatial and/or tactile conditional discrimination task.
Led to publications (1,3,4) and communications (1)
Fall 2008 - Spring 2010

**Pre-doctoral Student:** Griffin Laboratory: University of Delaware, Newark, DE: Utilizing in-vivo multi-unit electrophysiology to investigate the behavioral, anatomical, and physiological correlates of memory. Principal Investigator: Amy Griffin, Ph.D. Led to publication (2) and communication (2)

Fall 2007-Spring 2008

**Graduate Student:** Laboratory of Cognitive Neurobiology, Boston University, Boston, MA: Collected data for project investigating physiological characteristics of memory using in vivo multi-unit recordings of rat. Proficient in data analysis, surgical lesions, building and implant of devices for electrophysiology. Principal Investigator: Howard Eichenbaum, Ph.D. Led to publication (6) and communications (3)(4)(5)

Summer 2006 - August 2007

**Research Technician:** Laboratory of Cognitive Neurobiology, Boston University, Boston, MA: Administered complex behavioral tasks on rodents to distinguish the anatomical basis of recognition memory. Assisted in data and histological analysis. Principal Investigator: Howard Eichenbaum, Ph.D. Led to communication (5).

Fall 2004 - Summer 2006

**Research Assistant:** Dyslexia Research Laboratory, Beth Israel Deaconess Medical Center, Boston, MA: Performed histology, immunohistochemistry, western blot protocol, RNA isolation and autoradiography on rodent brain tissue. Principal Investigators: Glenn Rosen, Ph.D. and Albert Galaburda, M.D. Led to publication (5) and communications (7)(8)(10).

**Research Assistant:** Laboratory for Magnetic Brain Stimulation, Beth Israel Deaconess Medical Center, Boston, MA: Ran cognitive and procedural learning tests on human subjects. Principal Investigator: Daniel Press, M.D. Led to communication (9).

Fall 2003 - Fall 2004

**Student:** Developmental Neuropharmacology Laboratory, University of Massachusetts, Amherst, MA: Carried out experiments examining the behavioral and biochemical effects of MDMA on rodents. Principal Investigator: Jerrold Meyer, Ph.D. Led to publication (7) and communication (11).
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Summer 2003 - Spring 2004

**Research Assistant: Microbial Ecology Laboratory, University of Massachusetts**, Amherst, MA:
Assisted collaborative principle investigators researching microbial resistance to facially amphiphilic (flat hydrophilic/hydrophobic) compounds on various strains of bacteria. Principal Investigator: Klaus Nusslein, Ph.D. Led to publication (8).

**Behavioral Research**

Winter 2003 - 04

**Student: La Suerte Biological Field Station, Costa Rica:**
Designed and carried out three-week field study observing Capuchins (*Cebus Capucinus*) capabilities of self-recognition. Supervised by Anna Nekaris, Ph.D. Fall 2001 - Summer 2002

**Behavior Analyst: The New England Center for Children, Southborough, MA:**
Acted as an advocate and behavior-analyst integrating education with behavioral research paradigms for children with autism and other cognitive dysfunction.

**RESEARCH SKILLS**

- Histology and Stereology
- In vivo extracellular electrophysiology
- Immunohistochemistry
- Western Blot
- RNA Isolation
- $[^{35}S]$GTPgS Autoradiography assay
- Complex behavioral tasks for rodents
- Serial reaction time task for humans
- 5-HT Transporter assay

**PUBLICATIONS**


**COMMUNICATIONS**


**TEACHING EXPERIENCE**

2007-2008
- **Teaching Fellow**: *Boston University Graduate School of Arts and Sciences*, Boston, MA:
  - Led discussion sections, created and administered quizzes and exams.
  - Assisted Dr. Jacqueline Liederman in all aspects of undergraduate course: Physiological Psychology.

2002 - 2004
- **Faculty Advisor**: *National Youth Leadership Forum on Medicine*, Boston / San Francisco:
  - Facilitated group discussions in medical ethics and public health while emphasizing experiential learning in the medical field.

2000 - 2001
- **Instructor**: *Readak Educational Services, Inc.*, Acton, MA:
  - Facilitated a 12-hour advanced course emphasizing efficient reading habits for honors level middle and high school students. Taught in New York, Barbados, Puerto Rico and St. Thomas. Acted as sole representative for small international company handling all logistics including: scheduling, lesson planning, client recruiting, finances, and communications with administrators, faculty, parents, students and domestic office.
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COMMUNITY/ LEADERSHIP
December 2003 - present

**Student Member:** Society for Neuroscience and Federation of European Neuroscience

**Volunteer Counselor:** The Safe Haven Project, Fredrick, MD:
Assisted inner-city HIV-positive youth in constructive, outdoor activities.

5.4 PHD PORTFOLIO

Student’s name  Cullen Owens  
University  Erasmus University Rotterdam  
Department  ONWAR  
PhD duration  March 2010 - October 2013  
Supervisor  Prof.dr. Chris I. De Zeeuw  
Co-supervisors  Dr. L.W.J. Bosman  
Dr. M. Negrello

**General Courses**
2010  Laboratory animal science

**International conferences**
2009  Annual Meeting Society for Neuroscience (Chicago, USA)  
2010  FENS meeting (Amsterdam, The Netherlands)  
2010  Cerebellar Nuclei: Ins and Outs (Amsterdam, The Netherlands)  
2011  EU FP-7 CEREBNET: The cerebellum: from neurons to higher cognitive control (Pavia, Italy)  
2011  EU FP-7 CEREBNET Mid-term review (Jerusalem, Israel)  
2012  EU FP-7 CEREBENT Modeling the cerebellum (London, UK)  
2012  EU FP-7 Joint C7-CEREBENET (Barcelona, Spain)  
2012  FENS meeting (Barcelona, Spain)  
2012  EU FP-7 Joint C7-CEREBNET (Amsterdam, The Netherlands)  
2013  Gordon Conference on Cerebellum (New Hampshire, USA)  
2013  Annual Meeting Society for Neuroscience (California, USA)
5.5 Acknowledgements

Mom: this book is for you; your passion for life and love has given me so much hope in the world. Thank you so much for being a mom, friend, teacher and advisor. Dad: this book is also for you, your steadfastness, love, and patience has helped make me be who I am today. Thank you for always supporting me, I certainly couldn’t have done this without you both. I often think how lucky I am, to have been born into the family I was. Without you two, my choices might have been few and far between. Eva: you have always stood up for me, maybe even sometimes when you shouldn’t have. Thank you for helping guide me through this life. I am so lucky to know that we would still be each other’s support regardless of blood ties. You are the reason I often “highly recommend” big sisters. I will always do my best to be there when you need me. Matt: my bro! I always wanted a brother, and I have to say it has really worked out! I couldn’t wish for my sister to have a better man. And I couldn’t be luckier to have such a good friendship with him. Thanks for looking after my big sis, you two are a strong combination and you have both helped me so much in this phase in my life. Amelia and Chloe: this book is about your brain, you should use it! You two infuse an energy in me I forgot about. Thank you for reminding me. When your old enough, I’ll tell you some stories about how this thesis came to be. Mel: you have given me so much support that you will never understand. Thank you for being so many things to me. No matter how we lead our lives or how our lives lead us, you have and continue to be a source of strength and hope for me. Scott, Carter, Paul: I can only imagine our friend is looking down on us all with a stupid grin. It’s good to know you have friends no matter what. “The boys back home”: yes it’s true, you are reading something written by one of your friends who finally may be a doctor, not a real doctor mind you, but it’s something. I assume I will now dominate in poker, fantasy sports, witty banter or any other things that require strategy oh wait, I already do that. Thanks for being there as a group of friends I can always rely on. Kai: woah, dude, you have opened my eyes to so many things. I feel we are kindred spirits. Thanks for always listening and being there from the start of this. Don’t forget Pavia, Jersulem, fontainebleau, ciggins, and all our stupid jokes. Not many people are so lucky to have such a good friend during their PhD. Zee German: Jochen, you are such a rock, I remember having a beer with you discussing moving in together. I think my good karma allowed me to find you as a roommate. Thanks for the conversations late at night, Heineken in hand. By the way, it’s sometimes, not somewhen. These past few years have been anything but easy, but talking to you all on the phone and seeing you all has kept me strong.

Laurens: you guidance, patience, and calmness have helped me so much through this process. You are also a rock in our lab. Thanks for everything. Bas: Always there to lend an ear and a laugh, it can’t be understated how helpful that has been. Bendankt! Mario: Our conversations never get old to me. I was lucky to have you arrive when you did, on so many levels. Thanks for all the advice, and sometimes just the listening! Lieke: Thanks for all the
advice, for listening, all the ciggies and the ridiculous banter. Looking forward to more in the future! *Mafer:* So much has changed since you, me and Kai had drinks our first year. It has been great to have you as a friend and I look forward to watching Amaru grow older. *Carlos:* Thanks for all the good times, the good advice and akido training. You are a true master! *Yanzi:* I’m not sure how many lives you have lived in the past, but the wisdom is ever-present, you are close to my heart. *Rudiger:* Aarg! Exactly dude, exactly! *Brooke and Robert:* May many happy thanksgivings live on! *Taf:* Livin high and stayin smooth man. Good to always have a drink with you and looking forward to the next adventure! *Sander:* Your torch now bro! Jams at the studio, drinks in the office, and just shooting the breeze with you has been great! *Nouk:* You have lifted my spirits these past several months. Looking forward to new adventures. *Dr. Zandstra:* Froukje, you are a doctor in my book. Thanks for putting up with me for so long, I am so glad to see you moving on. You were one of the people who really made coming to work fun. *Pete:* Whiskey? Good times my friend, shootin pool, parties and BBQs, its been good to have you around. *Cindy:* go get’em girl. Thanks for all the hugs, high spirits, and strong spirits *Loes:* Ahhh, you hold the department together Loes, thanks for always brightening my day when I see you. *Kees:* Keep it real bud! *Paulo:* Thanks for all the good times, drinks at your place, my place, the factory, ha ha. *Elisa:* Thanks for all the food, advice, and science talks. *Corstiaen:* Thanks for hooking me up with the softball team man, can’t tell you how important it has been. *Jornt, Jan Willem, Henk-Jan, Martijn, Suman, Marcel, Michiel, Rogerio, Christos, Robert:* You guys have always been around, helping me keep my head above water, talking science and just keeping things fun and upbeat. I’ve gotten to know all of you a bit and I just want to say thanks. *Sara, Letizia and Licia:* You guys are great and have long careers ahead of you, keep up the good work! *Opher and Liya:* Thank you for being so cool. I got my professional advice from one and personal from the other, what an awesome combo! *Wei:* I was so happy you were in the lab. I know we both had some tough times, but we are stronger for it. Good luck with everything you do, and someday maybe I will be able to answer that question you asked me. *Negah:* Keep on truckin’ Negah! It was great to have you in the lab, to get to know you, to talk about the deep stuff. *Vincenzo:* Buddy, I can’t tell you how proud I am of you. Thanks for always valuing my opinion and knowing when to shrug off the crap life can give you. *Maarten:* I’ll never forget watching your talk on systems neuroscience, thanks for the hilarious facebook posts that keep my spirits high late at night! *Lena:* It has been great to get to know you recently, thanks for all the help with the thesis. I think the cover is beautiful. *Neptunus:* “Just hit!” *Aleksandra and Daan:* Congratulations guys, stay strong, and hope to see you around. Aleksandra, thanks for all the talks, your advice is always welcomed. *Chris:* when I met you in Chicago, you asked me what my greatest strength is and my greatest weakness. I told you I am creative, but sometimes I try to do more than I can handle. This thesis is probably evidence of that. Thanks for your guidance, your insight, knowing when to push, knowing when to let up, and
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leading me through the maze that is a PhD. I couldn’t have been luckier to have you as an advisor. This conversation was probably the pinnacle of that realization in an email which kept me laughing hysterically for about an hour: Chris: “You mean you are going to bed with your cell phone?” Me: “Ha ha, yes, unfortunately yes. It has its own pillow!!!! Whisker lab of the future: This book was actually written for you, a hippocampus guy who fell in love with the cerebellum. Don’t hate whiskers because we don’t have them, love them because we will never really understand them J. The department: When people ask you what you want to be when you grow up, nobody ever says, I don’t really care, as long as I work with good people; but they should. Trying to understand the brain is pretty fun, but it wouldn’t be without all the cool people who make it fun. Thanks for that! My scientific mentors; Jerry Meyer, Glenn Rosen, Howard Eichenbaum, Amy Griffin, and Chris De Zeeuw: I hope to take a little bit of everything from everyone, continue to learn to be a scientist with the aim to continue to learn about the brain. Thank you for helping make that possible. My Committee: I am truly honored to have you all present for my defense. I’ve never thanked anyone for giving me an exam, but I think it is fitting for this one; my last one.

I am certain I forgot many people. This is probably due to severe hippocampal degeneration, but I apologize anyway. If you don’t believe me I’ll show you my brain scan. There are so many people to thank I could probably write a book… I should do that… put it on the list!
Back Cover Illustration

Dear XXXX,

Please feel free to use any artistic, creative inspiration that comes to mind while in possession of this dragon. You can choose to change, add, erase, manipulate, color any part of the drawing. I will not edit or change anything from this point on. I have X weeks to finish my booklet. You will have X days to add your contribution to this drawing, which will be the illustration on the back of my booklet. If you choose for example, to modify/add to the structure, modify/add color, add teeth or simply darken or lighten contrast, anything and everything will be much appreciated. Just please keep in mind others will also contribute. The idea is to create an illustration that has no (or little) guidelines or controls, to add a metaphor to my project, a montage, that exemplifies some of the people and teamwork in my life here who have helped me accomplish my goals as a neuroscientist for me to remember. Therefore, for me, your contribution is an end in itself, and the subjective artistic impression it may have on others, is not of my concern.

All I ask is that you add to the illustration, and fit your initials into the illustration as well.

The person to whom this is addressed, the time left to finish my booklet, and the time I ask you to allocate to contribute, will be the only thing changed in this letter in order to eliminate any “bias” in the interpretation of the final published version. It will be sent to all who I ask to contribute. This message will also be published on the inset, but self-destruct shortly thereafter.

Thank you for enriching my life and my career.

Sincerely,

Cullen Owens
Epilogue

When I was 16, I wanted to be a photographer for National Geographic. My main goal was to save endangered species such as the Siberian tiger and the killer whale. I hoped by making people aware of the beauty in nature, that we as a species would curb our destruction of it. I majored in photojournalism at Boston University and during my first two to three years there, I found many disgruntled professors about the state of the media. Many of them had said the sensationalism taking place in reporting was diminishing the availability of unbiased information for society. My expectations of the path to get to my goal changed over that period, and I found myself falling in love with philosophy, changed my major and graduated with a bachelor’s degree in philosophy.

Over this period I knew my goals for a career changed from something specific to a general goal of having an impact on society while also enjoying the culture in which I worked. Six months after graduating from college I earned my paralegal degree and began working at an intellectual property law firm with the new hope of going to law school, and perhaps even becoming an advocate for the environmental protection agency or a district attorney. However, the firm I worked for specialized in biotechnology patents and our clients included scientists from Harvard University, Dana Farber Cancer Institute and the like. Our firm filed patents for the discoveries they made. I, again, worried about entering a field that would change who I am and what my values are, and I distinctly remember having had all my applications for ten different law schools neatly arranged in their envelopes but too hesitant to seal them and stamp them. I quit my job at the law firm, didn’t send out the applications and began making an earnest search for a career that fit my goals.

I then jumped around to many different jobs and fields, including teaching high school students, and working at a residential institution for children with Autism. At the same time I began reading books on the philosophy of neuroscience and was immediately hooked on the prospect of doing real empirical research. I also reflected upon my experiences and responses to these experiences and realized I was running away from fields because of the fear that I myself might easily lose my moral compass. The difference, I realized, was that rather than attempting to avoid such fields, it is more proactive to attempt to be an agent of change. I now know if one enters into a discipline fully aware of one’s goals and boundaries, it is possible to obtain those goals while staying true to one’s beliefs.

One of the reasons that neuroscience research is the best discipline that anyone could ever enter, is that it touches so many other disciplines. It can reach into everything from evolution, anthropology, and genetics to sociology, philosophy, psychology and even artificial intelligence and religion. However, we as scientists should always question whether the ends justify the means.
If we go into scientific research solely for the sake of satisfying our curiosity, the moral boundaries we set on ourselves may be limitless. My initial motivation to go into neuroscience was driven by curiosity and hopes of understanding the neural correlates of consciousness. However, my appreciation for the power and irony research in neuroscience holds, has grown since then. As current technology and methods for understanding the brain continue to advance at a break-neck pace, all of us in neuroscience, our community, we, are the only ones that can truly safeguard humanity’s interpretation of progress. Currently we are able to manipulate genes at specific locale in the brain, program insects to spy on our enemies, we can turn on and off genes and manipulate brain regions with literally the flash of a light. We are able to use animal neural signals to control robots, and we understand enough about the brain and pharmacology to subterfuge the processing of its traumatic experiences with the hope of bringing a higher quality of life for returning veterans with post-traumatic stress disorder. While curiosity will always be the fulcrum for discovery in scientific research, I hope to temper it with pensiveness.

Terms such as “technological progress” “higher order species” and “reason” are subjective expressions we use that explicitly interpret a species’ historical background. I feel honored to be a part of a community of biologists. And I will always be amazed at is magnificence. However, I am humbled by bacteria, which have no brain or attributes we consider to be inherently philosophical and yet can ground our interpretation of Darwin’s theory of natural selection. That is, it has adapted to its environment far longer than our species, without a single neuron. Therefore we should take care to consider survival in reference to the double bladed sword we call thinking.