Active perception of virtual texture frequency in the whisker-related sensorimotor system of the rat

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Tübingen, 28.01.2014 P.Georgieva, P. Georgieva

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Summary

Rats are able to sample their surroundings for tactile discrimination purposes by performing series of whisker deflections. In this perceptual state, called active perception, the animal chooses how to best manipulate its mobile sensors (the whiskers) based on the incoming sensory information it receives via these sensors, so as to perform better at the task at hand. On the other end of the perceptual spectrum lies passive perception which can be described as a “wake-up” call to investigate the surroundings if a sensation was received which was not expected by the subject. During active perception each deflection leads to a mechanical stress at the base of the whisker. Additionally, the touch of any object which the whisker finds along its path also leads to whisker deflections and hence whisker vibrations. These combined vibrations are termed the vibrotactile signal. The nature of the vibrotactile signal is highly complex and it is still not known which of its physical parameters are used by the animal for discrimination. Moreover, it is not yet clear if the tactile system of the rat integrates over this signal to reduce its complexity or if the animals have access to instantaneous kinematic parameters such as minute details of the whisker trajectory. Some of the possible parameters which the animals could use for performing tactile discrimination include the temporal frequency of the signal (i.e. its spectral information), its intensity (i.e. the mean speed of the signal) or instantaneous kinematic features (such as details of the whisker trajectory).

In this work I established a new psychophysical Go/No-Go paradigm for spatial frequency discrimination using actively whisking head fixed rats in a virtual environment where a natural stimulus is replaced by electrical microstimulation of the ascending sensory pathway. The major advantage of replacing natural stimuli such as textures with electrical stimulation is the greater level of stimulus control achieved from trial to trial.

The initial goal of the project was to investigate the differences in neuronal activation in the barrel and motor cortex arising in active versus passive perception. For the purpose I wanted to train animals to discriminate sets of virtual grids with defined line spacing first in an active case- where the animal has to sweep its whisker in space and receive an electrical pulse in the primary somatosensory cortex each time a grid line is crossed, and then in a passive case- in which the same animal is retrained to keep its whiskers still and the stimulation patterns from the active case are replayed in the cortex. To my great surprise, even after extensive training none of the experimental animals was able to discriminate between a set of two virtual grids with significantly different spatial frequencies. Thus I was unable to proceed towards my initial goal and show that the motor program of the animal would be changed in order to optimize sensory percept in active versus passive case.

Instead, I provided the animals with an additional cue, increasing the amplitude of the electrical stimulation on a No-Go trial as compared to a Go trial, which led to immediate discrimination for all tested animals. This finding speaks against pure frequency discrimination but rather indicates that rats are more able to use instantaneous events such as the electrical pulse amplitude cues. This new knowledge implied that the current working hypothesis that frequency is the most important parameter for rats during active or passive perception had to be revisited. I thus abandoned the pursuit to find the differences between active and passive perception, and concentrated on fleshing out if the instantaneous kinematic events as given by the electrical pulse amplitude were the actual parameter of a surface which the animals use for active discrimination. To thoroughly investigate this question, I varied several parameters of the virtual grids used in the active whisking task described here, including their spatial frequency, the electrical pulse amplitude and the position of the grid along the whisking cycle. I thus investigated an additional set of stimuli in which the electrical microstimulation amplitude in a Go trial was increased in even steps until it reached the value set for a No-Go stimulus. The stimuli providing an electrical pulse amplitude cue were easily discriminated by the animals whereas the stimuli differing only in spatial frequency were more challenging for the animals, leading to an increased amount of false responses. An experiment in which the virtual grids varied only in spatial frequency and starting position along the whisking cycle, thus blurring position information, proved the behaviorally most difficult one for the animals. The performance improved in a control experiment which kept the virtual grid arrangement same as in this experiment but provided the No-Go grids with electrical pulse amplitude cue. These results show that the rats were able to use the position information given by the virtual grids for discrimination. Altogether, one can conclude that the rats were not using the temporal frequency cues but used the electrical amplitude and grid position cue when available.

1. Introduction

1.1.Active/passive perception and surface parameters

Humans extract sensory information from their surroundings on a daily basis. Even the simplest activities, like pouring oneself a cup of coffee, require minute detail about the surfaces of objects, their form, weight and temperature. All of these sensory inputs, combined with feedback from visual and motor systems, allow the person to be successful in even the most complicated activities. Even though it has been the subject of innumerable studies, human sensory perception is still not thoroughly understood. Somatosensation can be subdivided in two modes- a state of passive perception and a state of active perception (Fig.1). Passive somatosensation can be desicribed as a “wake-up” call for a subject to explore its environment and understand the occurrence of an unexpected sensory stimulus. In this state the subject evaluates the incoming sensory stimulation in a mere bottom up fashion which does not lead to utilization of a proposed internal hypothesis, which holds the “world view” of the subject. The “world-view” of a subject is a description of the real world to which this subject has been exposed to and holds all the knowledge the subject has gathered throughout his life. As such, the “world-view” can also be described as the subject’s bias for interpreting any new incoming information which is not familiar- for example in biological mimicry a peacock butterfly which bears marks on its wings resembling predator’s eyes would be perceived by a bird as a predator, rather than as the butterfly it is. In an actively conducted perception, the subject has access to both the incoming sensory signals and his own internal hypothesis about the surroundings. For example, in a typical hunting situation a predator, which sees its pray and hence has all the incoming sensory information needed to locate and predict the future movements of its pray would be acting in an active perception manner modifying for example running speed or path trajectory such as to faster reach its pray. The pray on the other hand, as an example of passive perception, receives a sort of a “wake-up” call upon seeing the hidden predator on which it has to react if it wants to survive. In the state of active perception the subject should be able to use the incoming sensory information to perform better at the task at hand, i.e. tactile exploration. This could be achieved by modifying the neural activity in motor controlling areas of the brain and/or the areas holding the internal hypothesis based on the sensory feedback. Even though it is probably not directly related to an adaptation of the motor pattern, a rough switch between the states of active and passive perception has been shown to exist. The states of passive and active perception have been shown to lead to different activations of the neurons in the deep layers of barrel cortex (Hentschke et al., 2006). In this study an object was either actively touched by the palpating whiskers of the awake, head fixed rats or the stationary whiskers were passively deflected by the object, thus recreating the states of active and passive perception respectively. The authors found that active perception is described by lower response amplitudes to the object touch and elevated background firing as compared to the state of passive perception. Moreover, by transecting the infra-orbital nerve, which carries the sensory information from the whisker pad and whisker follicles, and delivering electrical stimulation via a cuff electrode to the caudal end of this nerve , the authors were able to show that the observed changes from active to passive state are of central and not peripheral origin.

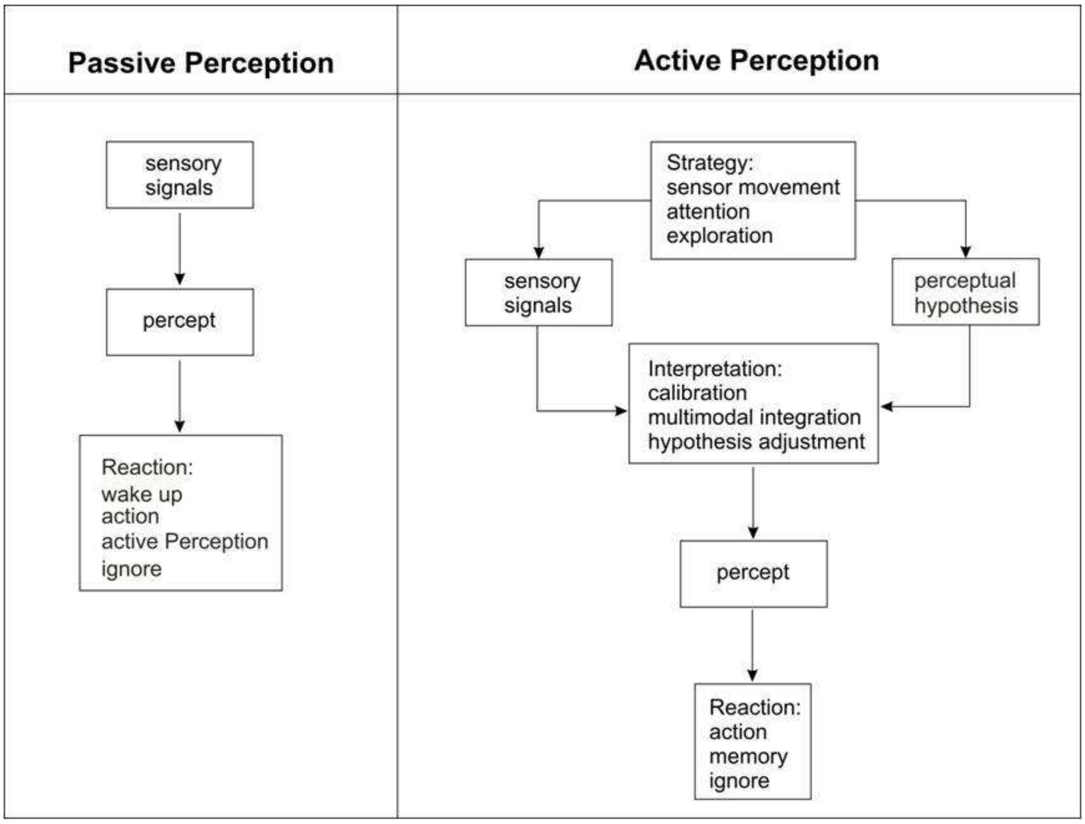

Figure 1 Active versus passive perception mode

Seminal work on sensory perception has proposed that the frequency contained in a surface is the primary parameter read out by the sensory system and used for further computation (Lamotte and Mountcastle, 1975), (Romo et al., 1998). In monkey discrimination experiments these authors claim to have been able to train the subjects to discriminate between stimuli varying only in spatial/temporal frequency. A mechanical sinusoidal stimulus was presented to the fixed hand of a monkey or a human, and a second comparison flutter stimulus varying only in frequency was presented either mechanically to the hand or as electrical stimulation pattern in the hand representation of primary somatosensory cortex. A flutter vibration is defined as the mechanoreceptive mode of somatic sensibility, evoked by mechanical sinusoids of 10-50 Hz delivered to the skin (Mountcastle et al., 1990), (Salinas and Romo, 1997).

In this experimental arrangement one can obtain insights into the state of passive perception, since the subject is confronted with the stimulus and does not have the chance to actively explore it. There are several candidate physical parameters which could be used by the sensory system for perception (Gerdjikov et al., 2010), (Waiblinger et al., 2013). First, events which exceed a threshold of high amplitude, velocity or acceleration can be extracted instantaneously from the vibrotactile signal originating in the skin during surface exploration. Second, the physical intensity of the tactile stimulus as described by its mean absolute velocity, kinetic energy or power can be extracted. And third, the frequency of the stimulus as described by the spectral centroid or by the number of cycles per second for repetitive stimuli can be extracted. These three possible physical parameters could not be investigated properly in the primate experiments described before. The use of sinusoidal patterns for stimulation does not allow the experimenter to disentangle the physical parameters of frequency and kinematic events, because these are interrelated. This problem has been addressed in the work of Salinas and colleagues (Salinas et al., 2000), as well as by Gerdjikov and colleagues (Gerdjikov et al., 2010) who used pulsatile stimuli whose intensity can be modulated independently by either modifying the inter pulse intervals (frequency) or the pulse wave form (the kinematic events). Another significant problem in the primate tactile perception literature as described so far is the missing active movement of the subject’s receptor (i.e. the monkey finger over the texture of interest) during the task. These studies try to deduce properties of the tactile sensory system based on passive application of sensory stimuli to the fixed hand of a primate. The rodent model system offers the possibility to more precisely investigate active perception since single moving whiskers can be tracked during tactile exploration with high temporal and spatial precision. Rodents, such as rats, which possess movable snout hairs called whiskers, are valuable test subjects in active sensory perception research. These nocturnal animals live subterranean and rely for navigation primarily on their whiskers (Vincent, 1912), (Ahl, 1986). In the laboratory they have performed very fine object and texture discrimination tasks using only the touch of the whiskers (Carvell et al., 1990), (Harvey et al., 2001). The head- fixed animal is of particular interest when the sensory stimulus is to be presented with constant stability (Schwarz et al., 2010). Head-fixed rats are able to perform well on detection or discrimination tasks with vibro-tactile mechanical stimuli delivered to the whisker (Gerdjikov et al., 2010). Even though this is a well controlled way of sensory stimulation it is not suitable for the investigation of active perception because of the passiveness of the whisker during the stimulation. Whisking in free air and simultaneous recording of the trajectories of one or all of the whiskers has already been established as a technique (Bermejo et al., 1996), (Bermejo and Zeigler, 2000). I went one step further and trained rats to perform a guided exploratory movement with one whisker in order to receive a sensory stimulus, which in my case was a virtual grid, composed of three lines. The virtual grid substitutes for a real grid and is realized by delivering a biphasic electrical pulse to the barrel cortex of the rat when the whisker crosses each of the lines of this virtual grid in space. This allows the conversion of the spatial frequency of the virtual grid into the temporal frequency of neuronal activation in the barrel cortex of the animal. The composition of the whisker movement was freely chosen by the rat and the virtual grid spacing was controlled by the experimenter. The main objective was to investigate if and how the animal modifies its whisking patterns when confronted with the different spatial frequencies of the virtual grids, i.e. if it whisks slower or faster when crossing a virtual grid of a lesser spatial frequency as compared to one with a higher spatial frequency. I trained head-fixed rats on a Go/No-go paradigm, tracked the movement of one single vibrissa with a laser sensor and delivered electrical stimulation to the primary barrel of this whisker based on its movement in real time. Electrical stimulation to the cortex has been shown to produce perceptual effects markedly similar to the ones obtained via natural (mechanical) sensory stimulation (Butovas and Schwarz, 2007), (Romo et al., 1998). It has also been shown to lead to neuronal sensory activations of barrel cortex similar to the activation observed when mechanical stimulation was applied to the whiskers (Simons, 1978a), (Butovas and Schwarz, 2003). The periphery of the rat's sensory system has a fairly complicated construction in which several sets of intrinsic and extrinsic muscles embedded in the whisker pad control the whisker movement (Dörfl, 1982), (Hill et al., 2008). Sensory signals such as vibrations of the whisker shaft induced by movement of the whisker or by it touching an object activate groups of mechanoreceptors situated at the base of the whisker inside its follicle (Arvidsson and Rice, 1991). The exact contribution of the activation of these receptors to the formation of sensory percept remains yet to be described. Electrically stimulating the primary somatosensory cortex allows us to circumvent this hurdle and to build a stable virtual sensory percept for the animal with each discrete electrical pulse in the barrel cortex mimicking the effect of an action potential emitted by the trigeminal ganglion upon a real whisker-object touch. Thus the virtual grids which I designed for this study differed in their spatial frequency, which was translated into the temporal difference of activation in the barrel cortex. The effects of the electrical microstimulation as delivered in our experiment are not unlike the synchronous activation of the sensory system when the whiskers are sweeping over a point like real structure, such as a grid made from rods. Moreover, the pattern of activity observed in barrel cortex after the whiskers touch a rod with single, short bursts of spikes followed by a period of inhibition (Hentschke et al., 2006) strongly resembles the pattern observed in barrel cortex after an electrical pulse microstimulation (Butovas and Schwarz, 2003), (Fanselow and Nicolelis, 1999). The manipulation of the amplitude, the frequency and the positions of the virtual grids achieved by using electrical microstimulation is comparable in precision to the one described in prior primate research. The use of electrical microstimulation as described in my experimental paradigm allows the investigation of active touch at a high precision which was not achieved in previous research in rodents or primates. The already mentioned similarities on both the neuronal as well as on a perceptual level between electrically stimulating the barrel cortex of a rat and mechanically stimulating its whiskers speak in favor of using electrical microstimulation for this work. The virtual grids consist of point-like virtual rods, resembling the vibrotactile stimulation using pulsatile stimuli and thus circumventing the problems occurring when using sinusoidal stimulation. Their delivery is made contingent on the movement of the rat’s whisker thus allowing us to investigate active perception. Altogether, the great advantage of this experimental set-up is the high degree of experimental control- from the whisker movement, precisely monitored in time and space, to the delivery of the sensory inputs by using electrical microstimulation.

1.2.Original experimental idea

A spatial frequency discrimination task based on virtual reality stimuli was designed in order to disentangle active and passive perception states. The task is based on electrical stimulation delivered to the ascending sensory system, which converts spatial parameters of the stimulus into temporal activation patterns of the sensory system. A set of easily distinguishable virtual reality grids is presented to the animal, which is supposed- after actively palpating over the virtual grid- to discriminate between a rewarded and a non-rewarded stimuli varying only in spatial, hence temporal frequency. The animal is free to choose a whisker movement pattern and actively samples the virtual environment. Once a stable discrimination level is reached in this state of active perception the animal is to be retrained to keep its whiskers stationary. Thus, the sensory system of the rat would be passively stimulated with the temporal patterns of activation responded best by the animal during active perception. Since the only parameter varying between the two states is the self generated whisker movement, which can be easily tracked and investigated, one can derive knowledge about the differences in the state of the animal during active versus passive perception. For both the active and the passive case a Go/ No-go behavioral paradigm was to be used to investigate the animal's percept.

A major set-back for this work was the finding that none of the experimental animals trained on the first step was ever able to reach a significant and stable level of discrimination on a Go/No-Go task in the active case when presented with pure temporal frequency cues for the discrimination. Thus, I was unable to move to the second step of the project and investigate passive perception. The work described here concentrates on investigating if the animals are at all able to manipulate the frequency of a stimulus such as to perform better at a specific task or if they prefer to use some other parameter of the stimulus, such as its overall intensity or the instantaneous events it provides. Based on the already mentioned inability of the animals to perform when only frequency cues were given, my new hypothesis was that it is rather the contribution of the instantaneous events to the animal's percept which needed to be further investigated in this work.

As a first step I needed to show that the inability of the rats to perform on the Go/No-Go paradigm as described here does not come from the general task design, i.e. the elements of the task that had nothing to do with the perceptual capabilities of the animal. For instance the rats had to learn to put their whiskers behind a specified starting point before issuing the whisk, and they had to obey waiting periods and periods free of licks, etc. Increasing the electrical pulse amplitude of the non-rewarded stimulus led to a major improvement in the performance of the experimental animals. This confirmed that that the established Go/No-Go paradigm is in fact, a task that the rats can learn, given they are able to perceptually discriminate the stimuli presented. This motivated the experiments described in this work, which try to answer the question which parameter of a sensory stimulus do the animals mostly take into consideration when actively palpating and discriminating objects in their surroundings- the frequency, the overall intensity or the instantaneous kinematic events.

1.3.Animal psychophysics and the Go/No-go behavioral test

Psychophysics tries to elucidate the relation between stimulus and sensation, usually by systematically varying the properties of the stimulus and investigating the arising percept of a subject. Animal psychophysics, as the name implies, searches to find answers comparable to the ones given by human psychophysics but also complemented with knowledge (usually invasively gathered) about the anatomy and physiology of the systems of interest. (Blough and Blough, 1977). Of main interest to us were psychophysical approaches which allow for a high degree of stimulus control and for stringent animal response readout. One such behavioral test is the Go/No- go paradigm (Table1).

Table 1 Go/No-Go paradigm response matrix for a two-stimulus set

stimulus response	present (predicting reward)	absent (predicting no reward)
present	hit (rewarded)	false alarm (punished)
absent	miss (unrewarded)	correct rejection (unrewarded)

Each trial of the Go/No- go test holds only one stimulus which has to be responded by the animal by a certain manipulation. If a rewarded stimulus was delivered the animal has to respond by eliciting a response (Go). If a non-rewarded stimulus was presented the animal has to withhold the response (No-go). In both cases the response manipulandum is always the same – i.e. a lick. A correct response to a rewarded stimulus (hit) leads to positive reinforcement (water) and a wrong response to a non-rewarded stimulus (false alarm) leads to negative feedback (tone, time delay for the begin of the next trial). All misses (no response to a rewarded stimulus) and correct rejections (no response to a non-rewarded stimulus) are not rewarded or punished and lead to the beginning of a new trial. The Go/No-go paradigm and its relatively easy concept allow experimenters to assess the animal’s percept in its entirety by presenting a range of stimuli to be discriminated to the animal. Drawbacks of this behavioral test are the non-balanced reward matrix, in which only a rewarded stimulus leads to an appetitive for the animal reward such as a drop of water and the possibility for a response bias to occur. A bias to elicit a response would lead to a higher proportion of false alarms to a non-rewarded stimulus. A bias to withhold a response would lead to more correct rejections but also to more misses of the rewarded stimulus. Another problem of the Go/No-Go paradigm is for the experimenter to tell apart misses from a disengagement of the subject from the task or false alarms from impulsivity. If used as a detection test the Go/No-Go paradigm should thus monitor the impulsivity and the motivation of the animal throughout the experiment (Schwarz et al., 2010) This can be accomplished by introducing “catch” trials and “reference” trials. In a catch trial, no stimulus is delivered to the animal thus allowing the experimenter to monitor how impulsive the test subject is to lick. A reference trial presents a highly supra-threshold stimulus to the subject, which it should be able to detect on each presentation, thus providing a read out for the motivation of the animal to work throughout each experimental session. Since “catch” or reference trials are not usually used with discrimination tasks which seek to find a measure of the ability of the animal to discriminate between stimuli and not to find the mere detection threshold for a stimulus, a possible way to overcome excessive licking of the rat is to introduce a longer inter stimulus interval, which could be reduced once the animal does not impulsively lick anymore.

In my Go/No-go discrimination paradigm I used so called “no activity windows, which were to be free of licks prior to stimulus presentation. In this window a lick leads to a time out, which acts as a punishment ensuring that no stimulus follows a lick by less than the duration of the time out. Also, I used time out as a punishment for any false alarms together with a tone as a feedback for the wrong response, to discourage impulsive licking.

The particular Go/No-go paradigm used in this work utilizes licks as the response parameter, water as the reward and a tone, light and time out as the feedback mechanisms. The head fixed animal is required to actively whisk and sample a set of virtual girds presented next to the snout across the experimental session. On each trial only one of the grids is presented, and based upon its prediction (rewarded, non-rewarded) the animal is supposed to emit a lick at a water spout or to withhold the lick. The discrimination parameter is the spatial frequency of the grids or a combination of spatial frequency and electrical pulse amplitude. The first correct lick to a rewarded stimulus leads to a water drop at the spout, and the first false alarm lick leads to a tone feedback. The movement of the animal's whisker which defines the beginning of a new trial is controlled by a light on/off cue. This Go/No-go test holds a certain memory component because there is only one stimulus on each trial, whose identity has to be decided against the other stimuli in the set.

1.4. The rat whisker system

The somatosensory system of the rat is one of the most often used systems in neuroscience research. In short, there are five arcs and four to six rows of whiskers on the rat's snout, and this arrangement can be observed as cell aggregations in sections of the brain stained for cytochrome oxidase on the level of the trigeminal ganglion (barrelettes), the thalamus (barreloids) and cortex (barrels) (Woolsey, T. A. and Van der Loos, 1970), (Welker and Woolsey, 1974) (Fig.2). The later name barrel cortex reflects the resemblance of the stained neuronal clusters found in tangential sections in layer IV of the somatosensory cortex to a barrel. Spaces between the barrels, which are not stained in a cytochrome oxidase section, are termed septa. The barrel cortex is strictly structured in the horizontal as well as in the vertical direction, with each barrel comprising a separate cortical column, delineated by septal columns. In the vertical direction the barrel cortex is organized in 6 layers, with the barrels residing in layer IV. Neurons residing in a specific barrel column tend to respond strongest and fastest to stimuli delivered to one specific whisker, which is termed the principle whisker (found on the contra lateral mystacial pad) and only to a lesser degree to stimuli delivered to adjacent whiskers (Welker, 1971), (Welker, 1976), (Simons, 1978b).

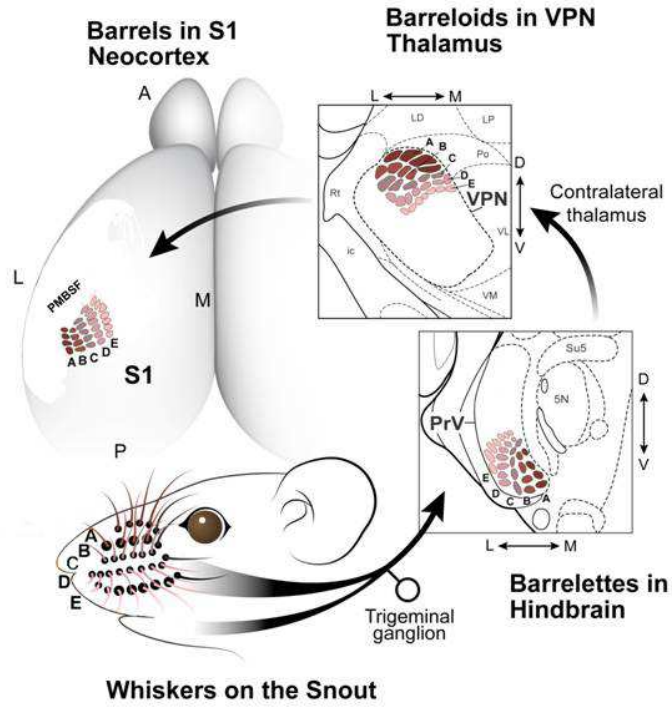

Figure 2 Arrangement of the rodent somatosensory system from periphery to cortex. Counterclockwise: whisker pad, barrelettes, barreloids and barrels (modified from (Zembrzycki et al., 2013)**)**

The neurons residing in septal columns have been investigated less thoroughly but are predominantly activated by multiple whiskers (Chapin, 1986). It is believed that barrel and septal columns comprise two separate information processing systems with different origins (Kim and Ebner, 1999), (Alloway, 2008). The trigeminal ganglion is the first station in the whisker sensory processing of the rat. It holds the bodies of cells which send axons towards the whisker follicle and towards the trigeminal nucleus in the brainstem. Neurons sitting in the barrels receive input from axons following the lemniscal pathway originating in the principal sensory trigeminal nucleus (PrV), which synapse on cells sitting in the ventroposteromedial (VPM) nucleus of the thalamus and from there reach cortex. Septal neurons on the other hand receive their input via the paralemniscal pathway- it originates in the interpolaris division of the spinal trigeminal nucleus (SPVi) and has its first synapse in the posteromedial nucleus of the thalamus (POm) and from there projects to neurons in the septal spaces and also to the barrel column layer Va and I. (Diamond et al., 1992). The major thalamic input to barrel cortex is in layer IV for the barrels and layers Va and I for the septal spaces, whereas axons of pyramidal neurons residing in layer V are the main output element of the system (McGuire et al., 1984), (Sherman, S M and Guillery, 1996), (Feldmeyer and Sakmann, 2000). The barrel cortex is highly interconnected with other cortical and subcortical areas, in particular with the primary motor cortex. Axons from the whisker representation in motor cortex project to septal areas in the barrel cortex, as well as to thalamus and to the facial nucleus in the brain stem, which is involved in generating the whisking movement (Miyashita and Mori, 1995).

Many other animal species are known for having whiskers on their snouts (the cat is a prominent example) but contrary to these species the rat has the ability to actively move its whiskers. The set of whiskers rats possess are not mere hairs but movable sensors on their snouts. A set of several intrinsic and extrinsic muscles (Dorfl 1981), (Berg and Kleinfeld, 2003) allows the animal to actively whisk and palpate objects and textures in its surroundings. Classical studies have shown that rats heavily rely on their whiskers for navigation and texture discrimination- animals deprived of the whiskers on one side of the face move along walls positioning the body site with the spared whisker set along the wall, avoid open spaces and are more prone to errors on water maze and other behavioral tests (Vincent, 1912). These studies were conducted using freely running animals, thus providing virtually no stimulus control to the experimenter. In a classical paradigm which again shows the degree to which rats rely on their whiskers to navigate the surroundings- the gap crossing test (Hutson and Masterton, 1986), (Barnéoud et al., 1991)- visually impaired rats or mice were asked to locate a distant platform and jump on it only relying on sensory input from the whiskers. Animals had to lean forward, whisk along the surfaces and make a decision. Even though elegantly showing that rats rely on their whiskers to process the surrounding, this experiment also does not give the experimenter much stimulus control. Later on head fixed preparations were implemented and single whiskers were tracked using optoelectronic whisker tracking (Bermejo et al., 1996), (Gao et al., 2003). The head fixed preparation, even though diverging from the natural state of the animal, gives the experimenter the opportunity to present highly controlled stimuli and to faithfully record the whisking activity of the animal during the test (Schwarz et al., 2010).

1.5.Electrical microstimulation in the barrel cortex of the rat

Naturalistic stimuli and the way the subject explores them are very difficult to control from trial to trial in an experimental paradigm. In order to overcome the limitations imposed to stimulus control by naturalistic stimuli I opted for electrical current as a stimulus of choice. Electrical current has been extensively used in neuroscience basic research as well as for the treatment of neurological diseases in the form of brain prosthetic devices. The electrodes delivering the electrical stimulation can be positioned in different parts of the brain dependent on the desired effect. Deep brain stimulation of the subthalamic nucleus, the globus pallidus interna or the periaquaeductal gray for example has been implemented for treatment of movement disorders such as Parkinson’s disease or tremor, or against chronic pain (Kringelbach et al., 2007). Electrical stimulation can also be used on the surface of the brain as a means to map the function of a specific portion of the cortex. Microstimulation has been used in attempts to restore motor functions (Barbeau et al., 1999), visual (Schmidt et al., 1996) or auditory (McCreery et al., 1992) percept.

Electrical microstimulation has been shown to predominantly activate axons passing through the area in the vicinity of the stimulation electrode (Butovas and Schwarz, 2003). The general notion is that the amount of electrical current injected through an electrode which is needed to activate a neuron is proportional to the square of the distance between the neuron and the electrode tip (Asanuma and Sakata, 1967), (Stoney, S D, Thompson, W D, Asanuma, 1968), (Yeomans and Tehovnik, 1988), (Jankowska and Roberts, 1972). The spatial spread of activation in the cortex when using electrical microstimulation has been investigated in a study by Butovas and Schwarz (2003). Even stimulus intensities close to the threshold for activating the barrel cortex in this study led to a spread of activation of up to 1.35 mm to each side around the stimulation electrode. It is known that if the electrical stimulation is delivered in the vicinity of an axon an action potential is generated which propagates along the axon. The initiation of the action potential can happen either in the cell body, the dendrites or the axon of the neuron (Stuart et al., 1997). Experimental and modeling studies have shown that the electrical stimulation mostly activates axons and rarely cell bodies or dendrites (Gustafsson and Jankowska, 1976), (McIntyre and Grill, 1999), (McIntyre and Grill, 2000). The activation order of the neural elements was investigated in slice experiments (Nowak and Bullier, 1998) and was based on the temporal properties of the elements such as rheobase, chronaxie and membrane time constants. Rheobase is defined as the minimal current of infinite time duration which results in an action potential. The minimum time in which a current twice the rheobase current is needed to produce an action potential is called the chronaxie. The membrane time constant determines how quickly current flow changes the membrane potential of the neuron. It depends on membrane properties of the cell, as well as on the geometrical arrangement of its elements in space. Via investigation of antidromic action potentials elicited by extracellular activation of the axons, intracellular current injections to the cell bodies or extracellular stimulation of the gray matter, these studies have shown that the chronaxie for axonal activation is up to forty times shorter than the chronaxie for a direct cell body activation, making the axon the first element to get activated via electrical stimulation in the vicinity of a neuron. These cronaxie measurements reflect the major differences between the cell body and the axon. Due to their large size cell bodies have higher capacitance than axons (Colombo and Parkins, 1987); they are also thought to have higher membrane resistivity (Major et al., 1994), as well as a lower sodium channel density (Ritchie et al., 1976), (Ritchie and Rogart, 1977), (Pellegrino et al., 1984).

In rats, single biphasic electrical pulses in the microampere range delivered to barrel cortex can elicit an artificial sensory percept mimicking the percept elicited by the whisker touching a real object (Butovas and Schwarz, 2007), (Dobelle and Mladejovsky, 1974), (Tehovnik, 1996), (Rousche et al., 2003). A disadvantage of this method is that it does not take into consideration any mechanics involved on the level of the whisker pad, the whisker follicle and the whisker shaft. However, I opt for using electrical stimulation because it allows for better stimulus control compared to using real surfaces as sensory input. It allows the manipulation of the frequency of the virtual grids and the amplitude of the electrical pulses independently of each other, comparable to pulsatile vibrotactile stimuli. It is also made contingent on the whisker movement, precisely tracked in time and space. Because of the already mentioned spread of activation in the cortex when using electrical microstimulation the effects of the electrical stimulation as used in my paradigm would spread over several columns of the barrel cortex, with an initial activation of the C1 column.

2. Methods

This work was performed on nine male Sprague Dawley head fixed rats used in preliminary experiments and three male Sprague Dawley head fixed rats used in the final psychophysical experiments, equipped with 2 by 4 mobile electrode arrays in the C1 barrel of primary somatosensory cortex for electrical microstimulation.

2.1.Pre-operative animal handling

All animals used for this study were obtained from Charles River at the age of 10 weeks and were accustomed to the experimenter for at least 2 weeks prior to surgical procedures. This phase was crucial for the consecutive training, as it helped diminish fear or stress of the animal. In short, during the first several days of handling animals were gently picked up, lifted and then released in their home cage until they were comfortable with this movement. Usually, signs of stress, such as vocalizations were never observed and the initial tense muscle tone of the animal upon touch was not seen after 2-3 sessions. Next, the animals were acquainted with the restrainer box and were let run freely through it in their home cage. For the animals the restrainer box was an object of novelty which all of them eagerly explored without any sign of stress, probably due to its resemblance to the natural habitat of the animal being a dark underground tunnel. Once a sufficient level of acceptance of the experimenter and the restrainer box was achieved, the animals were placed on a 2-day antibiotic treatment (Baytril® 2.5 %, oral solution, Bayer, 500ml), after which surgical procedures were performed.

2.2.Surgical implantation of electrode arrays

Animals were anaesthetized with 2% isofluran (Forene®, 250 ml, Abbott) and 90 mg/kgbw ketamin (Ketamin 10% (100 mg/ml), WDT) in medical Oxygen. Temperature was monitored with a rectal probe and set to 37°C. The fur on the skull was shaved and the skin was cleaned with sodium chloride solution. The head was fixed in a stereotaxic apparatus using blunt ear bars covered in Lidocain (Xylocain gel 2%, Astra Zeneca). For local pain relief Lidocain (Xylocain solution 1% Astra Zeneca) was administered subcutaneously along the midline of the head. After foot withdrawal reflex was gone, an incision was made in the skin along the midline; the skin was retracted to both sides covering the ears and was fixed with surgical thread. Lidocain (Xylocain solution 1% Astra Zeneca) was administered in the 2 lateral chewing muscles, the neck muscles and under the connective tissue covering the bone. Using a sharp spoon this tissue was removed and the lateral and neck muscles were gently detached from the skull. The exposed skull was cleaned with Hydrogen peroxide solution (3%, Otto Fischar) and sodium chloride solution (0.9%, Fresenius Kabi). The so cleaned skull was gently etched with Gluma Etch Gel (Gluma® Etch 20 Gel, 2.5 ml, Heraeus Kulzer) to obtain better adhesion of the dental cement in a later step. After having estimated the stereotaxic coordinates of the barrel cortex the desired positions of the miniature screws (J.I. Morris CO, US) were mapped on the skull surface. The screws were brought into place and the skull was covered with bonding agent (OptiBond FL, 2 component, 48% filled dental adhesive, Kerr) followed by layers of dental cement (Tetric EvoFlow, light curing, flowable, resin-based dental restorative, Ivoclar Vivadent). The area where electrode arrays were to be implanted and the positions of the screws used for grounding were left uncovered.

The position of the C1 barrel column in the primary somatosensory cortex was estimated using electrophysiological mapping and manual whisker stimulation. Multielectrode arrays were custom made. Eight pulled glass-coated platinum tungsten electrodes (80 µm shank diameter, 23 µm diameter of the metal core, free tip length ~10 µm, impedance > 1 MΩ; Thomas Recording, Giessen, Germany) were placed inside a polyimide tubing (HV Technologies, Trenton, USA), with distance between tips of about 300 µm, arranged in a 2 by 4 fashion and assembled in a microdrive fashion as described before (Haiss et al., 2010). The electrodes were soldered to Teflon-insulated silver wires (Science Products, Hofheim, Germany), which in turn were connected to a microplug (Bürklin, Munich, Germany). The array was brought into place, fixed using dental cement and protected by a custom made plastic tower from manipulation of the animal. A fixation screw was embedded head down in the rear part of the implant, on the bone covering the cerebellum. Silver LFP reference, animal ground and stimulation ground wires were positioned on the miniature screws above the cerebellum/olfactory bulb respectively and covered with dental cement. The whole implant was covered with silver conductive paint (Electrolube), skin was sutured and after administration of a pain medication ((Rimadyl Injection solution, 20ml, Carprofen, Pfizer), local antibiotic Nebacetin (15g Astellas Pharma) and 5 ml Glucose (Glucosteril 5%, 100ml, Glucose-Monohydrat, Fresenius Kabi) the animal was allowed to awake. Regeneration took at least 10 days with pain medication for the first 2 days after surgery. In the first week of the regeneration period the animals were given an antibiotic treatment (Baytril® 2.5 %, oral solution, Bayer, 500ml) Water and food were provided ad libitum.

2.3.Post-operative animal handling and head fixation

After complete regeneration, the animal was put on a water-controlled diet. Water was given to the animal only when successfully completing a task, such as running through the restrainer box or accepting holding the head screw by hand. After several sessions most animals tolerated head fixation for several minutes. Once this level was reached, the animal was transferred to the training set-up and water was delivered using a built-in water spout.

2.3.1. Classical and operant conditioning

The first step of the training was association of the water spout with a water reward. Water drops were presented to the animal through the water spout only if a randomized interval free of licks has passed. At the beginning of the training this interval was around 2s. The animal learned to lick at the spout in order to collect the water reward and to suppress excessive licking which would otherwise delay the water drop presentation. The association between spout and reward was almost immediate. Next, a train of electrical stimuli (5-15 biphasic electrical pulses, 30-50 µA) was delivered to the barrel cortex simultaneously with the water reward. Gradually the number of electrical pulses was brought down to one, the interval that had to be free of licks was increased up to 5s and the water reward was now delivered only if the animal successfully detected an electrical stimulus by licking at the spout in the 1s interval after stimulus presentation. Next, the active whisker movement was conditioned to acquire the electrical stimulation. To do this the C1 whisker was inserted in a polyimide tube and tracked at ~ 2 cm away from the snout using a Metralight laser sensor (Bermejo et al., 1998) (Metralight Inc., San Mateo, USA) with high temporal and spatial precision allowing whisker tracking in real-time.

Whisker position was then tracked in real time (with a frame rate of 0.4 ms), and whenever the animal brought its whisker behind a specified starting position (usually the resting position of the whisker) the house light went off to signal the start of a trial in which the animal then had to move forward to receive an electrical stimulus at the C1 representation of the barrel cortex. At this point special care had to be taken to assure that the animal kept associating the stimulus to reward and not the movement: Firstly, movements which occurred outside a specified time window did not lead to stimulus presentation. Secondly movements started with house light on (i.e. whisker on the wrong side of the starting line) did not yield any stimulus presentation. After the whisker was placed in the starting range and the house light went off the animal had 1s to perform a whisk. Gradually the distance between the start and goal position (at which electrical stimulation was applied) was increased until a full amplitude whisk was reached covering the Metralight sensor extent of 28 mm. Up to now single pulse stimulation was applied whenever the whisker reached a certain protracted position. Now this stimulation was replaced by a grid consisting of three spatial points, each of which triggered the application of a single electrical biphasic pulse to the barrel cortex whenever the whisker passed the point in forward direction (protraction). In this way the spatial virtual object – so to say a 3-line spatial grid - was translated into a temporal series of three electrical pulses delivered to the barrel cortex. The final goal of this training was to have the animal working on a Go/No-go paradigm discriminating between a set of stimuli varying in spatial frequency or spatial frequency and electrical pulse amplitude. The animal had to whisk across the virtual grid presented in each trial and based on its identity (Go or No-Go grid) to lick or to withhold the lick at the window of opportunity.

2.3.2. Importance of the spatial frequency parameter in virtual texture discrimination

After successful conditioning to active sampling of virtual grids, as explained above, all experimental animals went through a set of behavioral tests designed to answer the question if the spatial frequency of the grid is the physical parameter on which rats base their active tactile percept. The concrete set of experiments was the subject of an extensive set of preliminary experiments in which all task parameters were optimized. Importantly, in these preliminary sets of rats, the strategy of the tests was adapted to the fact that rats had great difficulty to discriminate spatial frequency alone (this was the reason that the animals could not be confronted with pure spatial frequency cues from the start, because then they could not learn the complex psychophysical task).

2.3.2.1. Task 1- Spatial frequency and electrical pulse amplitude cues discrimination

The first behavioral experiment was used to estimate the ability of the rats to discriminate stimuli that differed in pulse amplitude and spatial frequency. A set of 2 discriminanda was presented in a 1:1 ratio: a rewarded stimulus (Go) with low spatial frequency (f) and low electrical stimulus amplitude (a) was presented on 50% of the trials, and a non-rewarded stimulus (No-Go) with high spatial frequency (F) and high electrical stimulus amplitude (A) was presented on the other 50% of trials. The stimulus matrix was as follows (Table 2):

Table 2 Virtual grid and electrical stimulation parameter matrix for task 1

	Go	No-Go
Virtual texture frequency (f)	low	high
Electrical pulse amplitude (a)	low	high
Abbreviation	fa	FA

The biphasic electrical pulses used had duration of 500 μs and amplitudes as follows (Table 3):

Table 3 Electrical stimulation parameters for task 1

	Go (μA)	No-Go (μA)
Rat 1, Rat 3	30	150
Rat 2	50	200

2.3.2.2. Task 2- Spatial frequency cues discrimination

After reaching a stable discrimination performance on this task the animals received a new set of stimuli: 4 rewarded stimuli (Go 1 to Go 4) and one non-rewarded stimulus (No-Go). The amplitude of electrical stimulation increased from Go 1 to Go 4 in equal steps, so that the last one (Go 4) would have the exact same amplitude as the No-Go. In particular these were as follows: a1 (for Go 1), a2 (for Go 2), a3 (for Go 3) and a4 (for Go 4). As mentioned, a4 equaled A, which was the amplitude used for the No-Go stimulus. The spatial frequency of all 4 rewarded stimuli was equal and was lower than the spatial frequency of the non-rewarded stimulus (Table 4). Thus, by design, Go 1 and No-Go were identical to the stimuli presented in subtest 1, whereas Go 4 and No-Go differed only in spatial frequency, *but not* in electrical pulse amplitude. This test provides information about the ability of the animal to take frequency as the discrimination parameter in a sample of stimuli. The four rewarded stimuli together comprised 50% of the trials in an experimental session with No-Go providing the other 50% of trials.

Table 4 Virtual grid and electrical stimulation parameter matrix for task 2

	Go 1	**Go 2**	**Go 3**	**Go 4**	**No-Go**
Virtual texture frequency (f)	low	low	low	low	high
Electrical pulse amplitude (a)	a1	a2	a3	a4=A	A
Abbreviation	f a1	f a2	f a3	f A	F A

The biphasic electrical pulses used had 500 µs duration and amplitudes as follows (Table 5):

Table 5 Electrical stimulation parameters for task 2

	Go 1(µA)	**Go 2(µA)**	**Go 3(µA)**	**Go 4(µA)**	**No-Go(µA)**
Rat 1, Rat 3	30	70	110	150	150
Rat 2	50	100	150	200	200

2.3.2.3. Task 3- Spatial frequency and temporal (positional) cues discrimination

Next, I designed a reversed set of stimuli with one rewarded stimulus (Go) and four non-rewarded stimuli (No-Go 1 to No-Go 4). In this subtest all stimuli (rewarded and non-rewarded) had the same electrical pulse amplitude but differed in spatial frequency and in absolute position on the whisker protraction path. The rewarded stimulus had a lower spatial frequency than the non-rewarded stimuli, which in turn had exactly the same spatial frequency from No-Go 1 to No-Go 4 but were positioned displaced to one another, with No-Go 1 being the most dorsal and No-Go 4 the most rostral on the whisker protraction path (Table 6). Thus, the four No-Go stimuli introduced an uncertainty about the absolute position of the grid, which earlier could have been used to do the discrimination. Here No-Go 1 and 4 would begin or end where the Go stimulus began and ended. The other No Go stimuli were located in between. Together the four No Go stimuli comprised 50% of the trials whereas the single GO stimulus was presented in the other 50% of the trials.

Table 6 Virtual grid and electrical stimulation parameter matrix for task 3

	Go	**No-Go 1**	**No-Go 2**	**No-Go 3**	**No-Go 4**
Virtual texture frequency (f)	low	high	high	high	high
Electrical pulse amplitude (a)	low	low	low	low	low
Abbreviation	fa	Fa	Fa	Fa	Fa

The electrical pulse amplitude in this task for the all the stimuli was as follows (Table 7):

Table 7 Electrical stimulation parameters for task 3

	Go (μA)	**No-Go 1 to 4 (μA)**
Rat 1, Rat2	100	100
Rat 3	150	150

2.3.2.4. Task 4- Spatial frequency, electrical pulse amplitude and position cues discrimination

The last task was used as positive control and utilized the same stimuli as task 3 but provided an additional electrical pulse amplitude cue. All non-rewarded stimuli (No-Go 1 to No-Go 4) had now higher stimulation amplitudes as the rewarded stimulus and had the same absolute positions on the whisker protraction path as the stimuli in task 3 (Table 8). The ratio of rewarded stimuli to non-rewarded ones was again 1:1.

Table 8 Virtual grid and electrical stimulation parameter matrix for task 4

	Go	No-Go 1	No-Go 2	No-Go 3	No-Go 4
Virtual texture frequency (f)	low	high	high	high	high
Electrical pulse amplitude (a)	low	high	high	high	high
Abbreviation	fa	FA	FA	FA	FA

The electrical pulse amplitude for rewarded and non-rewarded stimuli in this task was as follows (Table 9):

Table 9 Electrical stimulation parameters for task 4

	Go (µA)	No-Go 1 to 4 (µA)
Rat 1	50	200
Rat 2	50	100
Rat 3	50	150

2.4. Data Analysis

Whisker tracking and real-time electrical pulse delivery were accomplished using National Instruments LabView RealTime software. All data analysis was performed offline using custom written Matlab software (Version 7.9.0.529, R2009b, The MathWorks, Inc.). The velocity of the whisker was obtained by differentiation of the raw whisker position trace. Whisker velocity distribution was calculated for all whisker protractions in the 1s stimulation interval of the active whisking task. Also, exact whisker velocity upon cross of each grid line was calculated and presented as a distribution over the 1 s stimulation interval. The number of whole grid crosses in each trial was calculated and plotted as a distribution over all behavioral sessions of the respective test and with respect to the response of the animal (hit, miss, correct rejection or false alarm). The resulting distributions for both the number of grid crosses and the respective velocities were plotted using box whisker plots. In short, a box whisker plot describes data distributions by providing medians (central mark on the box plot), 25th and 75th percentile values (edges of the box plot), extreme data points still considered as a part of the distribution (box plot whiskers), as well as any outliers (asterisks). The distributions of grid crosses were analyzed for significant differences with respect to the behavioral response of the animal using pair wise independent-samples Kruskal-Wallis non-parametric test (IBM SPSS Statistics 21). Same analysis was performed also for the distributions of the whisker velocity.

3. Results

In the course of this doctoral thesis work I trained head fixed rats to whisk in free air and to perform a Go/ No-Go discrimination task based on the perceived spatial frequency difference between virtual grids of three lines delivered by means of electrical microstimulation in the C1 whisker column of the barrel cortex. In a first step of the training, all animals were conditioned to respond faithfully to passive electrical pulse microstimulation. Single biphasic electrical pulses (30-50 µA) were delivered to the C1 barrel on a randomized time interval schedule and the task of the animal was to lick when it detected an electrical pulse. All rats achieved significant levels of detection after 5-6 sessions in average (Fig.3)

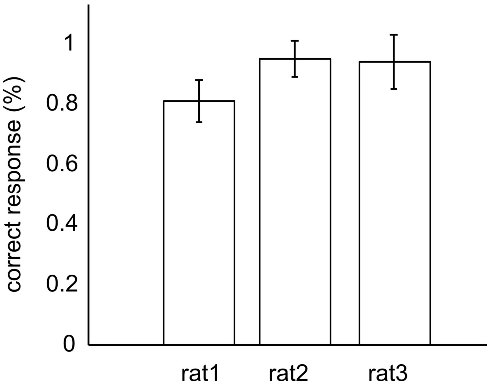

Figure 3 Percentage correct responses for all experimental animals over all sessions with passive electrical pulse stimulation (biphasic electrical pulses, mean ± standard deviations)

The active whisking part of the training started once a robust detection of the passive electrical stimulation, without excessive spontaneous licking was achieved. The movement of the rat's C1 whisker was tracked using a MetraLight sensor, read out with LabView Real Time software and used for a real-time electrical stimulation based on the whisker position in each specific time point. (Fig 4A)

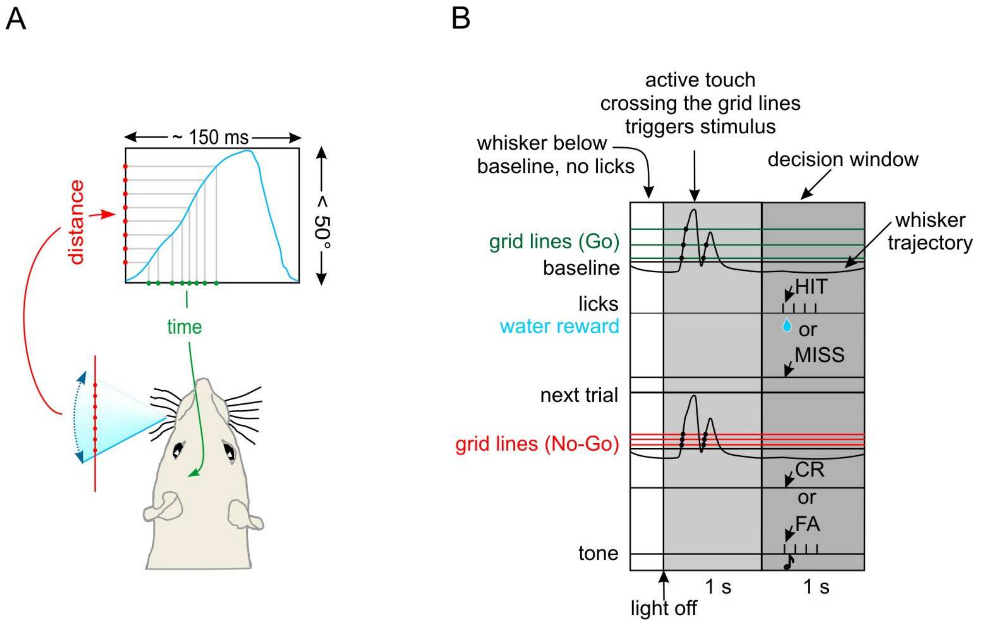

Figure 4 A: conversion of virtual grid spatial frequency into temporal frequency of electrical stimulation in the barrel cortex; B- Go/No-Go task schematics (a false alarm leads to a waiting period for the duration of the tone (2s) not schematically depicted here)

The electrical stimulation was delivered only on a protraction movement of the whisker and upon crossing a line of the virtual grid. The animal’s task was to start a trial by positioning its whisker behind a virtual baseline and withholding any licks for ~200ms. (Fig 4B) When this condition was met, an indicator light was turned off and the animal had 1s time to perform a movement of its own choice and to sample the presented virtual grid. A trial was counted as valid if at least one full crossing of the whole virtual grid had been done in this 1s interval. The animal then had 500 ms time to indicate its response by a lick to a water spout if a rewarded stimulus was perceived and withholding the lick to a non-rewarded stimulus. The performance of the animals was estimated for each training session by calculating a discrimination index (di). The discrimination index was calculated by subtracting the relative number of false alarms in a session from the relative number of hits.

In task 1 I investigated the ability of the animals to discriminate virtual grids varying in spatial frequency, as well as in electrical pulse amplitude. The electrical pulse amplitudes used for the pair of rewarded/non-rewarded stimuli differed between rats (Table 3).

All three rats achieved significant levels of discrimination on task 1 already in the first 3-4 training sessions (for the three rats: di in the range 0.84 to 0.94) compare Fig 5 B and D, condition 1). The discrimination indices for all training sessions during task 1 are presented in Figure 5B and D. For task 1 the animals accomplished 13, 12 or 10 sessions respectively, with on average 85- 160 trials per session.

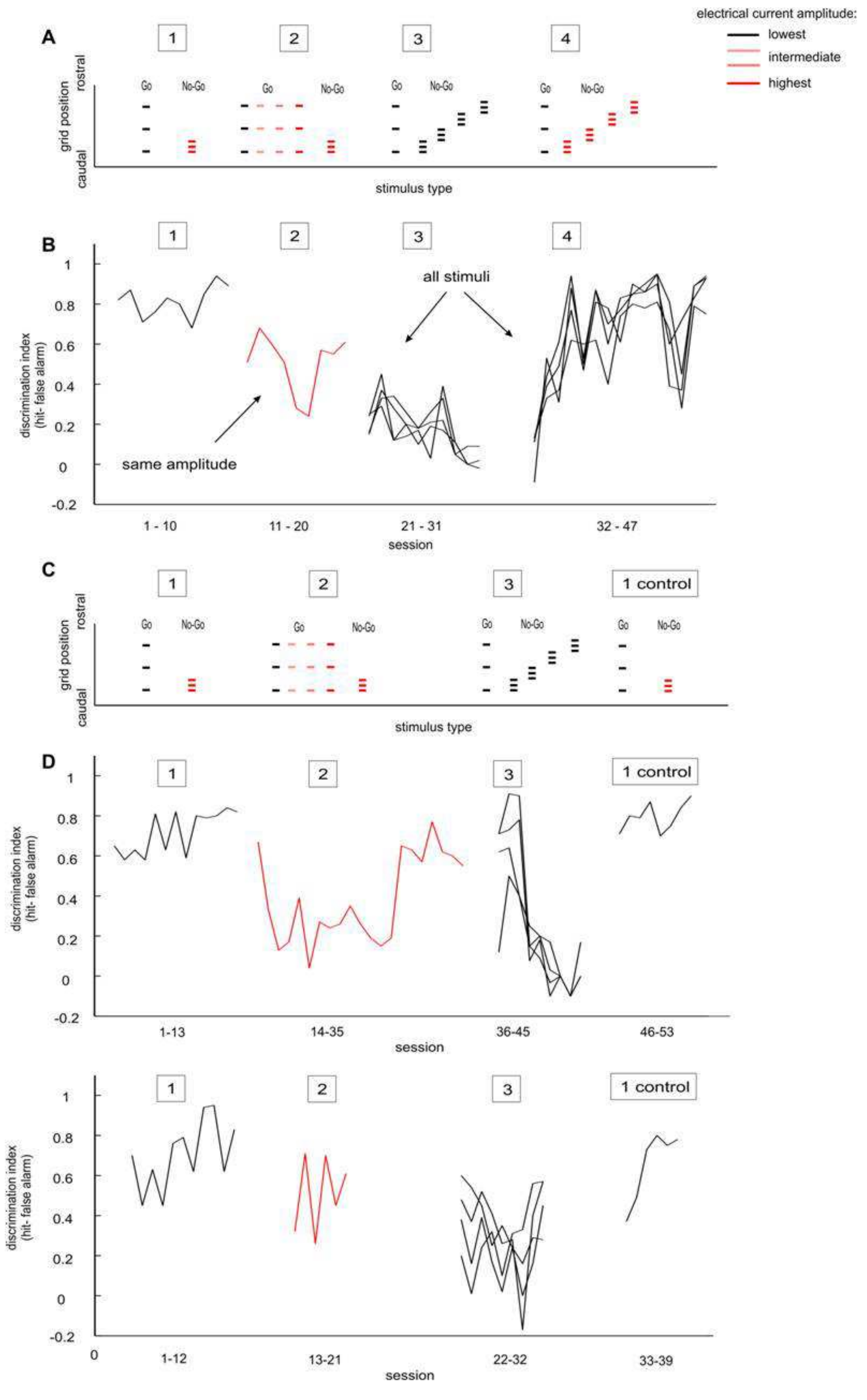

Figure 5
Psychophysical Results

A Stimulus set: virtual grids differing in spatial frequency and/or electrical pulse amplitude B: Discrimination index evolution for all of the experimental conditions (for test 2 only the discrimination index between the rewarded and non-rewarded stimuli matching in stimulation pulse amplitude is plotted). Line plots represent all behavioral sessions in the respective test condition; C: stimulus set for the other two animals ; D: Discrimination index evolution for these two animals

In task 2, which investigates the ability of the animal to discriminate using frequency cues, (Fig. 5 B and D, condition 2) I introduced a set of rewarded stimuli with equal spatial frequency but increasing electrical pulse amplitude and a non-rewarded stimulus with a higher spatial frequency and electrical pulse amplitude matching the one for the fourth rewarded stimulus (Table 5). In this task the impulsivity of the animals increased. The consequence was that they appeared to correctly respond to the rewarded stimuli but failed to correctly reject the non-rewarded stimulus on the majority of experimental trials. (for the three rats: di1 in the range 0.5-0.77, di2 in the range 0.66-0.77, di3 and di4 in the range 0.68-0.77). In task 2 the animals accomplished 21, 10 and 9 sessions respectively with on average 11-21 trials for each non-rewarded stimulus and 55-100 trials per rewarded stimulus.

In task 3 (Fig. 5B and D, condition 3) I investigated if the animals take the absolute start and/or end position of the No-Go grid in space, rather than the spatial frequency cue as the basis for the discrimination. A set of four high spatial frequency No-Go stimuli was introduced in this task (Table 7). The location of these stimuli was distributed in between the start and end virtual line of the Go stimulus with No-Go 1 being the most caudally positioned one and No-Go 4 the most rostrally positioned one. In this task the animals performed on 10, 9 and 10 sessions respectively; with on average 45-87 trials per session for the rewarded stimulus and between 10- 18 trials per session for each of the non-rewarded stimuli. The performance of all three experimental animals dropped even further (for the three rats: di1 in the range 0.34- 0.64, di2 in the range 0.45- 0.91, di3 in the range 0.37- 0.78, and di4 in the range 0.29- 0.5).

This result suggests that some of the remaining performance of the animals had been due to the unique position cue in task 2. Blurring this cue by positional variation in task 3 made them perform even worse. This result speaks in favor of the hypothesis, that the animals could not use the frequency cue, but were able to use the position cue at least as long the position cue was constant across trials.

Task 4 utilized the same discriminanda as task 3 but with re-introduced electrical pulse amplitude cues for the non- rewarded stimuli. This task provided the subject with electrical pulse amplitude cues missing in task 3. The discrimination indices for the three rats were: di1 in the range 0.56-0.81, di2 in the range 0.7- 0.93, di3 in the range 0.7-0.95 and di4 in the range 0.7-0.95. During this experiment one of the three animals was trained on a task with four NoGo stimuli (Table 9, Rat3). It was able to return to a level of discrimination comparable to the ones observed during task 1 or task 2 (as presented in Fig 5B). This animal performed 15 sessions on the task 4-schedule with an average of 84 rewarded stimuli per session and 17 trials per each of the non-rewarded stimuli presented. The other two animals were retrained on task 1 using 50 µA electrical amplitude for Go and 200 µA for the No-Go stimulus. Here the di mean values were 0.80 and 0.71 and di max 0.9 and 0.8. These animals performed 6-8 sessions on the control-task schedule with an average of 50-66 trials per rewarded and non-rewarded stimulus.

In short, from the first psychophysical experiment one could conclude that the rats were able to discriminate well between stimuli with different electrical pulse amplitude and different positions. In the second experiment, taking away the frequency cue for one of the discriminanda, the performance of the rats was worse than in the first experiment, but the animals were still able to discriminate the two stimuli. This discrimination ability could be due to the different positions of the two grids on the whisking cycle. Once this positional cue was deteriorated by positioning the non-rewarded grids at different positions on the whisking cycle (in test 3) the discrimination ability of all animals was severely diminished. The fourth experiment, which provided the blurred positional cue together with an electrical amplitude cue, showed that the animals can use the electrical amplitude cue for discrimination while whisking. This control experiment points towards a more important role of the electrical pulse amplitude for discrimination as compared to the position or frequency cues.

In further analyses, I investigated the whisking strategy of the animals used to solve each of the tasks. I compared how often the animals would cross each virtual grid in the 1 second time interval they had to sample the grids and what their whisker velocity was upon crossing each of the lines of a virtual grid. In the example trace presented in Fig 6 the animal crosses the rewarded virtual grid 3 times during the stimulation period.

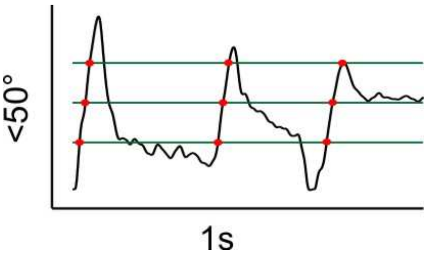

Figure 6 Trajectory of the C1 whisker during a crossing over a rewarded grid. Green lines represent the virtual grid, red dots specify the time of electrical stimulation, black line is the movement of the whisker. An upward movement along the black line represents protraction of the whisker and a downward movement along the same line represents retraction of the whisker.

For task 1 I found no consistent pattern for a change in the grid crossing numbers based on the virtual grid identity. There was only a slight trend that the animals would sample the non-rewarded virtual grid less often (1 to 2 crosses on average) than they sample the rewarded grid (3-4 crosses on average, Fig.7 A). The same was also true for the velocities with which the whisker crossed each grid (Fig.7 B). The velocity for a rewarded grid crossing in two animals was found to be slightly higher than for a non-rewarded grid. The medians of the velocity distribution for a hit were 518 and 561 mm/s, whereas the respective medians for a correct rejection lay at 477 and 496 mm/s. The third animal which crossed the rewarded and non-rewarded grids in a more uniform fashion (median of the grid cross distribution for a hit was 4 times and for a correct rejection 3 times) displayed median velocities of 533 mm/s for a hit response and 548 mm/s for a correct rejection response. I found very few consistent differences for the velocity profiles during this test including a slight difference for a “hit” versus “correct rejection” trial for all animals and a slight difference between a CR and FA trial in only one animal (Bonferroni-adjusted p-values for comparing velocities in a “hit” versus “correct rejection” response were p= 0.005, 0.001, 0.004 and p= 0.009 when comparing CR versus FA response).

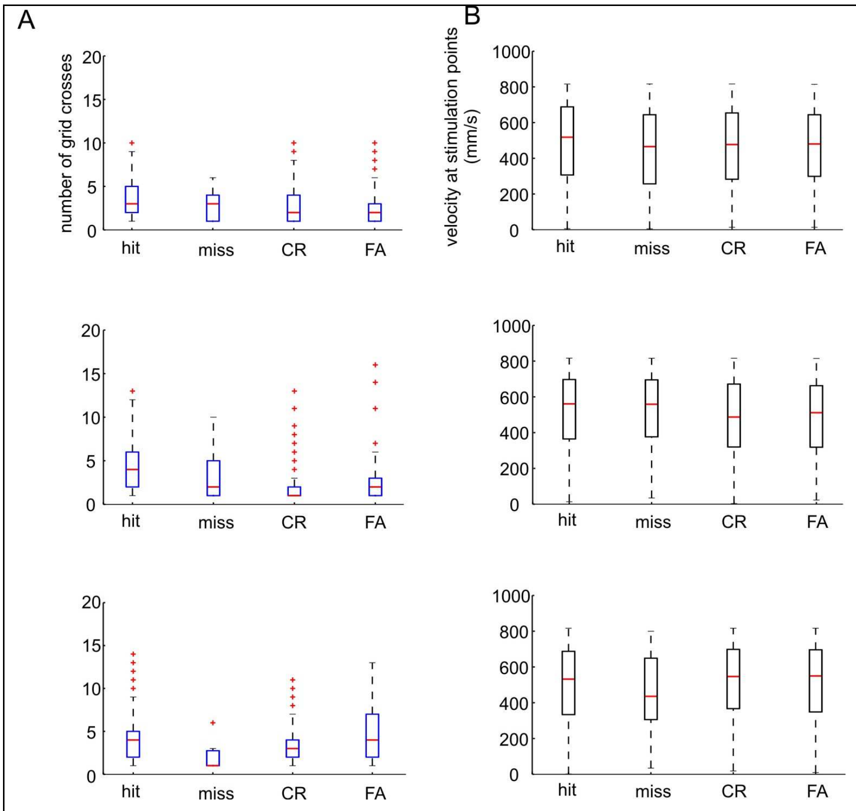

Figure 7 A: Box-whisker plots of the number of grid crosses during all sessions of task 1 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and

the outliers are plotted separately with red crosses.
B: Box-whisker plots of the velocities with which the whisker crossed the stimulation points of the grid during all sessions of task 1 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses.

In task 2 the trend that all four rewarded grids were also sampled more often than the non-rewarded grid was also present (8±3 grid crosses for rewarded grids and 3±1 grid crosses for a non-rewarded grid, Fig. 8 A). There was no difference in the number of grid crosses for a correct rejection versus false alarm. The velocity distributions changed only slightly throughout animals and behavioral responses (Fig 8 B). One of the animals performed the task using a stereotypical whisker velocity (median of the distributions was in the range of 530-570 mm/s) which was not statistically different for any of the possible responses. A second animal also used a stereotypical whisker velocity (medians of 520- 550 mm/s) which varied only when crossing the first of the four rewarded grids, which also had the least stimulation intensity. This grid was crossed with velocities similar to the velocities used for a non-rewarded grid (medians of 234 and 275 mm/s respectively). The third animal crossed the rewarded grids with velocities slightly higher than the velocities used for a non-rewarded grid crossing (median 510 and 580 mm/s respectively, Kruskal-Wallis non-parametric test $p<0.001$). For none of the animals did I find a statistical difference in the velocity when comparing a correct rejection versus a false alarm response.

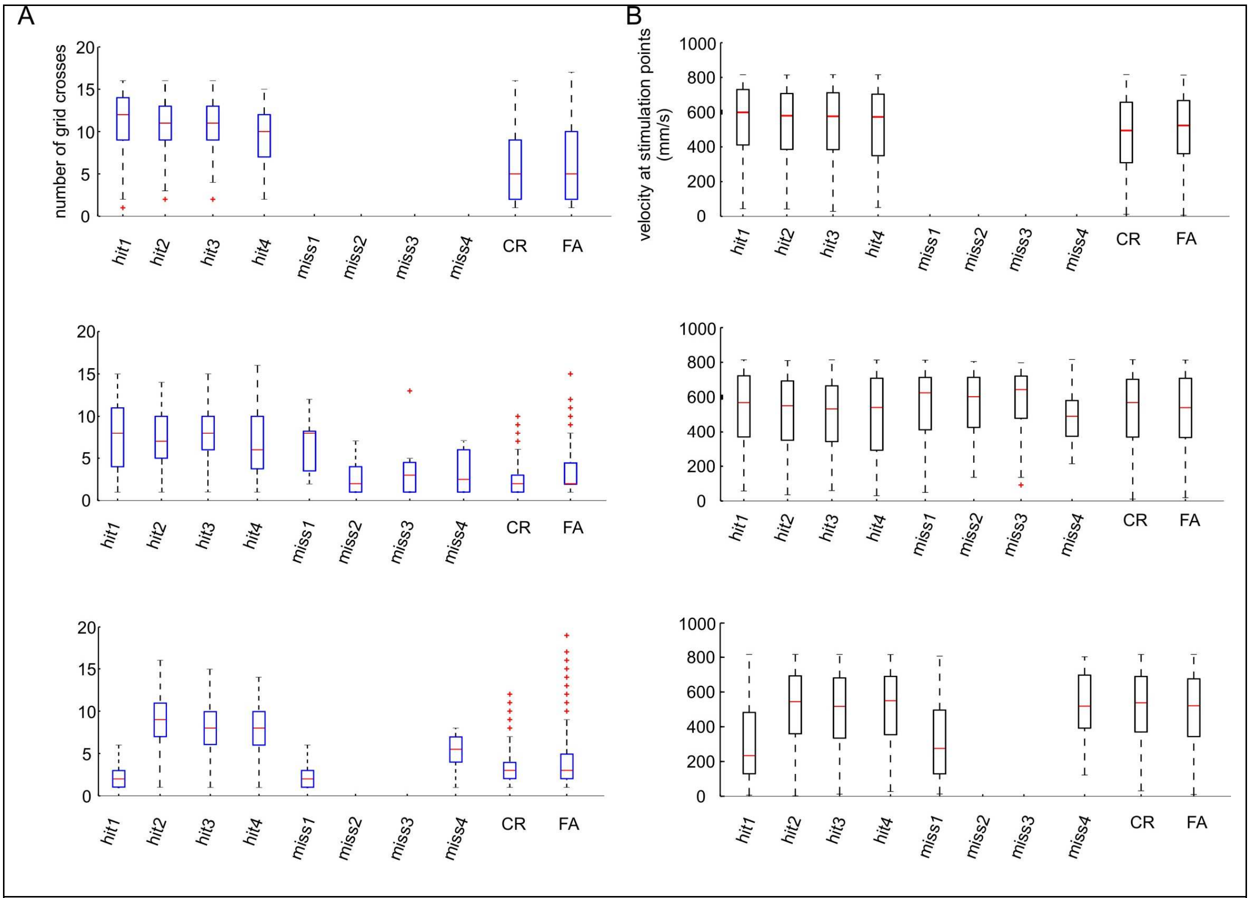

Figure 8 A: Box-whisker plots of the number of grid crosses during all sessions of task 2 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses. The missing box-plots in the "miss" cases indicate 100 percent detection rates for the rewarded stimulus

B: Box-whisker plots of the velocities with which the whisker crossed the stimulation points of the grid during all sessions of task 2 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses. The missing box-plots in the "miss" cases indicate 100 percent detection rates for the rewarded stimulus

The blurred positional cues and the absence of the electrical pulse amplitude cue in task 3 forced the animals to use diverse whisking strategies. One animal crossed the rewarded grid and the most rostral of the non-rewarded grids equally often (in median 5 times, but chose to sample the other three non-rewarded grids less often in median 1-2 times, Fig. 9 A). Another animal crossed the rewarded grid equally often as the three most rostral non-rewarded grids (median 5 times) but chose to cross the most caudal non-rewarded grid less often (1-2 times).The third rat crossed the rewarded grid equally often as the two most extreme non-rewarded grids (median 3 times) but crossed the two intermediate non-rewarded grids more often (median 7-8 times). However, these varying whisking strategies did not lead to markedly different behavioral outcome, since the discrimination indices were the lowest observed during all experiments for all three rats. There were only some minor variations observed in the velocity distributions (Fig. 9 B). One of the animals used a stereotypical whisking velocity which did not differ between the various possible behavioral outcomes (450 mm/s in average for a rewarded grid and 460 mm/s for a non-rewarded grid). A second animal crossed the most caudal of the non-rewarded grids with velocities higher than the velocities used for crossing the rewarded grid (median of the velocity distribution for a hit was 420 mm/s, for a correct rejection 520 mm/s, and for a false alarm 490 mm/s, Kruskal-Wallis non parametric test p= 0.026 for a correct rejection and p= 0.003 for a false alarm response). This was the only animal which displayed a statistical difference in the whisker velocity distributions for a correct rejection versus false alarm for the third most rostral non-rewarded grid (median of the velocity distribution was 470 mm/s for a correct rejection and 375 mm/s for a false alarm, Kruskal-Wallis non parametric test p=0.034). The third rat crossed the rewarded grid with velocities higher than the velocities used for the non-rewarded grids (median of the velocity distribution for a hit was 593 mm/s and ranging from 460 to 540 mm/s for a correct rejection). These observed differences in the velocity distributions were so small and inconsistent that I feel they do not reflect any biologically significant phenomenon, but are more representative for the small sample size of this experiment.

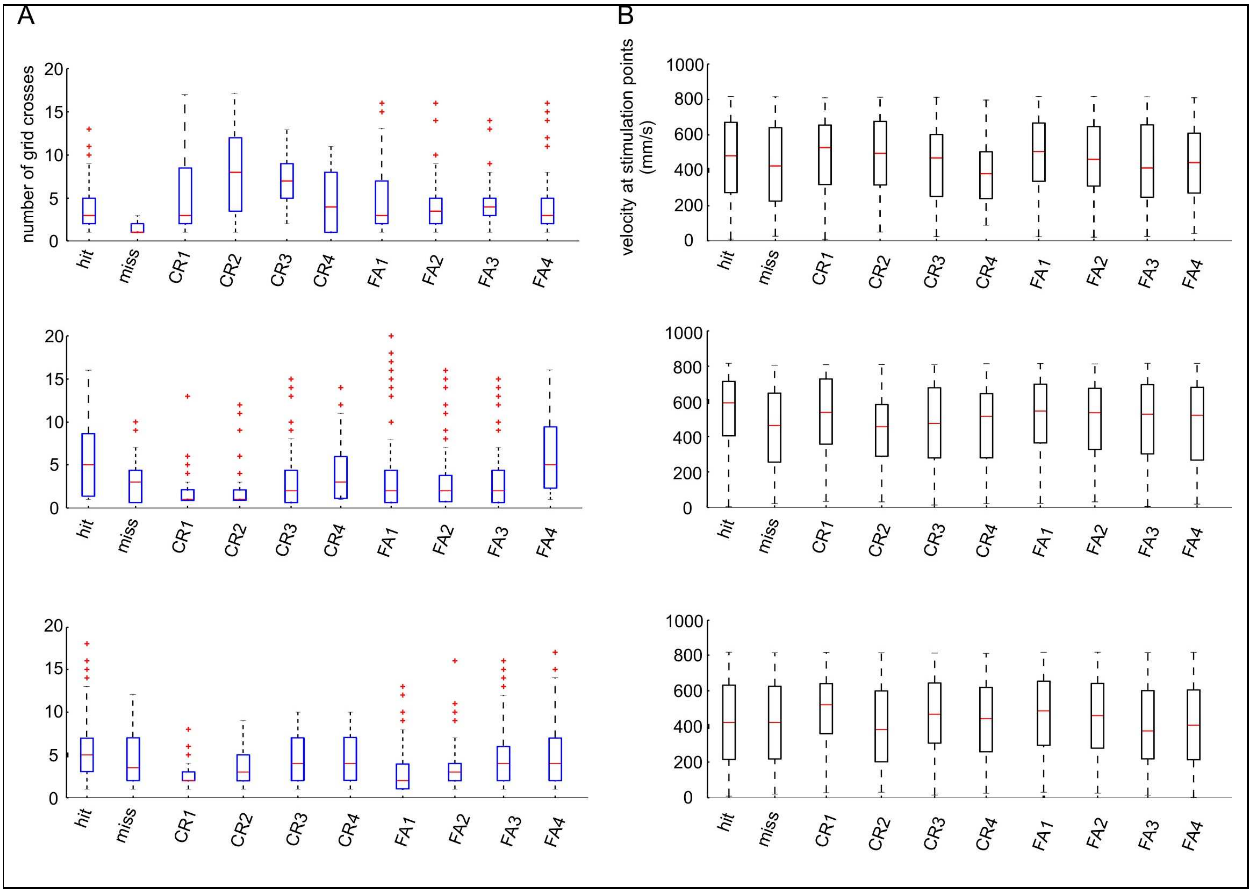

Figure 9 A: Box-whisker plots of the number of grid crosses during all sessions of task 3 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses

B: Box-whisker plots of the velocities with which the whisker crossed the stimulation points of the grid during all sessions of task 3 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses.

Only one animal performed well in task 4, which used the same set of stimuli as task 3 but providing additional electrical pulse amplitude cue for the non-rewarded stimuli. Interestingly, this animal tended to cross the most rostral non-rewarded grid the most often (comparable to the number of crosses it performed on a rewarded-grid, 9±2 versus 8±2 times, Fig. 10 A). The other three non-rewarded grids were sampled less often 3±1, 4±1, and 5±1 times respectively. There were only negligibly small correlations between the velocity with which a whisker crossed a virtual grid and the behavioral response of the animal (Fig. 10 B).

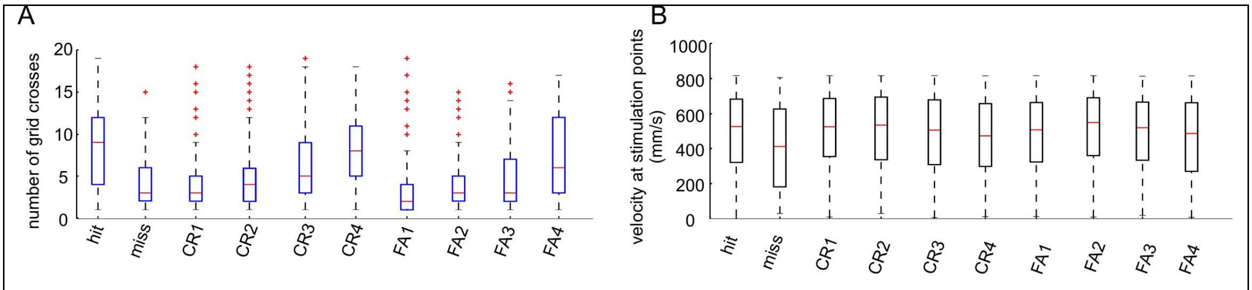

Figure 10 A: Box-whisker plots of the number of grid crosses during all sessions of task 4 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses.

B: Box-whisker plots of the velocities with which the whisker crossed the stimulation points of the grid during all sessions of task 4 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses

Data for the animals which did not perform well on test 4 is given in the Supplementary Fig.1. These two animals were retrained on test 1 with success levels comparable to the levels reached at the beginning of training. For these animals I found some inconsistent variations between the grid crossing patterns as well as some negligible variations in the whisking velocities (data in Supplementary Fig. 2).

There was a slight trend that with increasing the task difficulty the animals would try to sample the virtual grids more often. Moreover, in two of the animals I observed an increase of the absolute amplitude of a whisk coupled with a high whisking speed. These movements, which the animals started performing during task 3, which was the most difficult discrimination task, resembled foveal whisking with frequencies up to 15-20 Hz. Interestingly, this change in whisking strategy did not prove helpful for the performance and the discrimination index level were lowest for task 3. During task 4, which provided additional electrical amplitude cues, one of the animals abandoned this energy- costly strategy and returned to its original whisking strategy, whereas the second animal continued using the high whisking strategy. These animals were not able to use the offered intensity cues optimally, probably due to the frustration level which they experienced in the previous task. Nevertheless, once presented with a fairly easy to discriminate stimulus set such as the one in the control experiment (repetition of task 1, with one Go and one No-Go stimulus with different spatial frequency and different electrical amplitude) both of the animals returned to discrimination index levels similar to the ones in the beginning of the training.

The investigation of the licking behavior of all three rats during the different tasks showed that the animals tend to increase the false alarm rates with increasing task difficulty. Figure 11 shows the response probabilities for each rat and each stimulus set. In task 1 the licking responses to the No-Go were low for all three rats, whereas in task 3, the most difficult one for all three animals, the responses to a No-Go stimulus were significantly higher and approached the response level for a Go stimulus. For the one animal which performed task 4 with frequency and electrical amplitude cues the false alarms were at a level comparable to the level in task 1. Same observation was true for the other two animals which performed the control experiment. From the lick response patterns shown here one can conclude that the psychophysical performance shown in Figure 5 strongly depends on changes in the false alarm levels. Figure 11 shows high hit levels for all rewarded stimuli in the different tasks and for all animals. The discrimination index as presented in Figure 5 is calculated as the difference of hits and false alarms. Thus, increasing the false alarm rate while keeping the hit performance constant leads to lower discrimination indices, and vice versa.

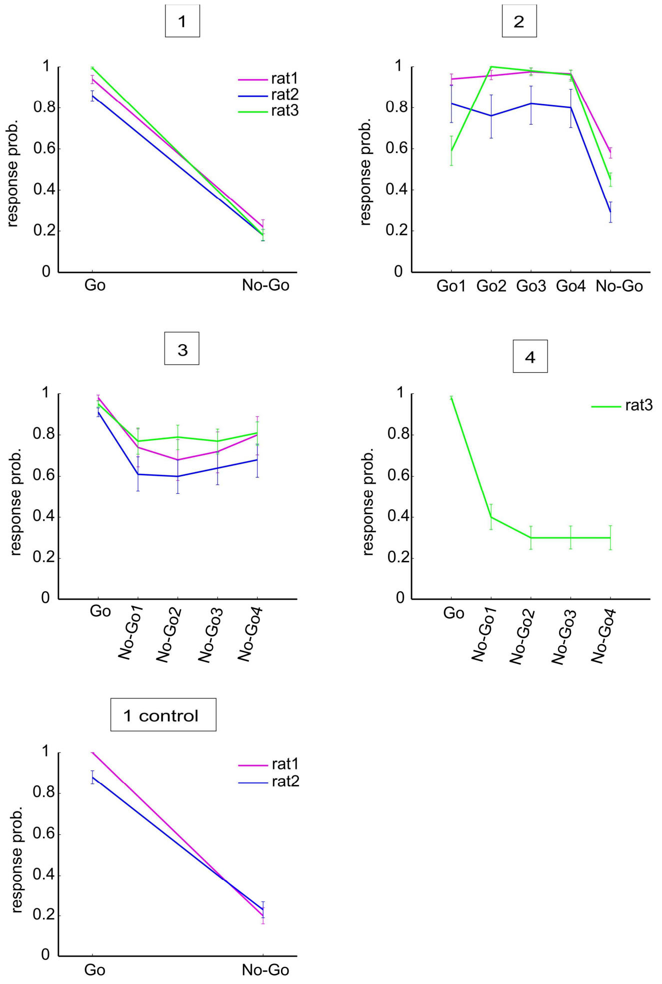

Figure 11 Lick response probabilities

Mean lick response rates for all sessions of the different behavioral tests and all animals (error bars: 95% confidence intervals). False alarm rates increase with behaviorally more challenging tasks.

4. Discussion

How are we able to tell the difference between a scarf and a paper tissue when going through a bag without looking at its contents? This deceivingly trivial question which everyone can answer by “Well, they feel different” still does not have a precise scientific answer. What parameter of these very different surfaces do we actually “feel”? How are the mechanoreceptors in our skin activated by these different textures while our hands go over them, and what does our cortex make out of this incoming information?

These questions have been addressed in seminal primate experiments, postulating that the frequency of the surface is one of the main important parameters the sensory system cares about (Lamotte and Mountcastle, 1975), (Mountcastle and Romo, 1990). With few exceptions, (see (Gamzu and Ahissar, 2001)) primate literature had mainly investigated the state of passive perception and it is difficult to conclude from these experiments how the important surface parameters might change between the state of passive and active perception. Moreover, by using sinusoidal stimuli of varying frequencies the authors not only changed the frequency but also the intensity (calculated as the mean absolute velocity) of these stimuli. The frequency and the wave form of a sinusoid are interrelated and changing one definitely leads to changes in the intensity of the stimulus. This problem does not apply to pulsatile stimulations in which the frequency or the form of the pulse can be independently modified. By using such pulsatile stimulations at the base of a rat’s whisker Waiblinger and colleagues (Waiblinger et al., 2013) were able to show that instantaneous cues in the vibrotactile signal are a primary candidate used by the animal during passive whisker deflections. The finding that whiskers and human skin are both bioelastic elements whose vibrations add instantaneous kinematic cues to the vibrotactile signal based on the surfaces at hand (Scheibert et al., 2009), (Jadhav and Feldman, 2010) suggest that these cues might very well be present, but overlooked, in primate perception.

In the course of this thesis work I tried to investigate if the instantaneous kinematic cues are also used during active perception. Several parameters of the virtual grids used in the active whisking task described here were varied, including their spatial frequency, the electrical pulse amplitude and the position of the grid along the whisking cycle. In the first experiment the animals received spatial frequency cues, electrical pulse amplitude cues and positional cues (task 1). The discrimination levels reached in this task were the highest reached during this study. Next, I introduced a pair of stimuli having the spatial frequency and the positional cues, but delivered with the same electrical pulse amplitude (task 2). Here, even though there was a decrease in the discrimination index, the animals were still able to discriminate between these virtual grids. A possible explanation for this finding is that the rats were actually able to use the positional cue if forced by the experimental design. When this positional cue was made less reliable by introducing an array of non-rewarded grids (task 3) with different starting positions along the whisking cycle, all rats failed to discriminate. By introducing electrical pulse amplitude cue for these non-rewarded grids (task 4) the discrimination performance recovered. These results suggest that the animals were able to most reliably use the information provided by the electrical pulse amplitude cue. The deliverance of the electrical stimulus to the cortex based on the instantaneous position of a whisker in space is comparable to the instantaneous changes in the whisker position used by Waiblinger and colleagues (Waiblinger et al., 2013). Recordings of primary afferents in rats (Jones et al., 2004a), (Jones et al., 2004b), (Shoykhet et al., 2000), (Stüttgen et al., 2006), (Chagas et al., 2013) as well as from all stages of the ascending sensory system (thalamus, (Petersen et al., 2008)); barrel cortex ((Pinto et al., 2000), (Stüttgen and

Schwarz, 2008)) showed that fast kinematic changes in the whisker trajectory are faithfully represented by spikes with high temporal precision. Even though it has not been explicitly investigated in my work, I assume that the activation of barrel cortex achieved by the electrical microstimulation would be comparable to the natural activation of the cortex during a whisker sweep over a real grid of three lines. Electrical microstimulation of the barrel cortex has already been shown by Butovas and Schwarz (2003) to generate a stereotypic pattern of activation, starting with a fast and short excitation followed by a longer period of inhibition. The amplitude of the electrical pulse was shown to have similar effects to a repetitive stimulation. The increment of the electrical pulse amplitude, similar to bigger pulse or ramp- stimuli, generates bursts of activity in the cortex with higher number of spikes.

It is interesting that the proposed instantaneous cues seem to be used by the sensory system in both the passive perception state (investigated by Waiblinger et.al) and in the active sensation case, as described here. This finding speaks in favor of instantaneous events being of utmost interest for the somatosensory system of the rat, irrespective of the perceptual state. It also comes in contrast to the existing primate literature which postulates that frequency is the most important surface parameter used for discrimination. In studies by Hernandez and Romo (Hernández et al., 2002), (Romo et al., 1998), (Romo et al., 2000) the experimental monkey was confronted with a discrimination between a mechanical sinusoidal stimulus pattern applied to the skin of a finger tip and electrical stimulation pattern delivered directly to the cortex. As a first step towards this test the authors trained the animal to discriminate the frequency of two sinusoidal mechanical vibrations delivered to the finger tip. In the actual experiment comparing the mechanical stimulation and the electrical microstimulation, the authors modified the amplitudes of their electrical stimulation pulses such as to match the subjective intensity perceived by the animal from the mechanical stimulus. A problem with this experiment was the fact that the authors did not asses the subjective intensity curves of all their individual test subjects but used the subjective intensity curve estimated in a first experiment for all subsequent ones. Even though it is labor-intense, one should investigate the subjective intensity curve for each individual separately as these may significantly vary between subjects.

Interestingly, (Lamotte and Mountcastle, 1975) also adjusted the amplitudes of their mechanical stimuli so that the perceived intensity was equal to a comparison stimulus and used this set-up to test the ability of humans and monkeys to discriminate stimulus frequencies. The amplitude of these sine waves at different frequencies was adjusted such as to correct for subjective intensity. Doing so, the authors generated sine waves with very different peak velocities and amplitudes. These could potentially have been used by the animal for the discrimination task instead of the assumed frequency differences. Thus, the percept arising in the somatosensory system of the subjects cannot be ascribed with certainty to a single stimulus parameter, such as the amplitude or the frequency of the stimulus.

When a comparison between pure frequency differences is sought to be made electrical microstimulation is a good method of choice. It has been successfully used to produce a sensory experience mimicking the natural one (Salzman et al., 1990), (Romo et al., 1998), (Cohen and Newsome, 2004), (Thomson et al., 2013), (O’Doherty et al., 2012). Because the spike patterns evoked by our virtual grids resemble those of pulsatile deflections they are free from the dependency of frequency and waveform known from sine waves. Like the mechanical pulse in a pulsatile deflection the electrical pulse (and its effect) is independent from pulse frequency, and thus, can be modulated independently from it (Waiblinger et al., 2013).

The head-fixed active whisking rats used in this study did not seem to modify their whisking with increasing task difficulty. Crossing a static virtual grid with different speeds would produce also different neuronal activation patterns in the barrel cortex and would lead to a markedly different sensory experience, which could potentially be helpful in a discrimination task. This was however not the strategy used by the experimental animals, not even when there were no electrical stimulus amplitude cues. If one imagines the whisker movement in the horizontal direction around a pivot point on the snout, then in task 2, by design, the rewarded and non-rewarded stimuli have a fairly different arrangement. The non-rewarded stimulus is situated in the most caudal part of this region of whisker movement and the virtual lines of the rewarded stimuli are spaced equally over the region of whisker movement. Thus, the first stimulation point for the rewarded and non-rewarded stimuli lies on the same part of the whisker trajectory, but the second and the last points of stimulation are different. This arrangement provided both spatial frequency cues and positional cues for the animal. As shown in task 3, spatial frequency is not used by the animals, thus most of the discrimination performance seen with task 2 must have come from the positional cues. Thus, due to these very salient sensory cues the animals were able to perform well on this task while actively whisking, a behavior which goes a step further than what mice showed in a previously described pole localization task, (O’Connor et al., 2010). In this Go/No-Go test, the animal had to localize the occurrence of a vertical pole in a target or distracter position and discriminate between the two positions by emitting a lick if the pole was in a target position. Even though the authors claim that mice perform this task using active whisking, it is actually the pole which descends into the reach of the whiskers on each trial. This very artificial stimulus design is in clear contrast to my task in which, even though presented as a virtual reality, the rat encounters a very well spatially defined texture on each trial. O’Connor and colleagues state that “mice predominantly explore the region of the rewarded stimulus and avoid the no-go stimulus”, referring to a possible strategy of the animal to solve the task. What is a more probable explanation is that these mice were actually performing a detection task by estimating the presence or absence of a pole. Due to the different task design of my experiment the rats were actually prevented from doing what the mice of O’Connor and colleagues did and actually used whisking to locate the virtual objects. This shows that rodents are capable of using whisking signals for object location and rather use the best possible solution given a specific task than using a stereotypic strategy.

Based on the behavior observed during the different tests described in this thesis, one can conclude that the rats did use the positional cue to perform the discriminations, but were faced with a major challenge when this cue was made more obscure by varying the starting positions of the stimuli (task 3). The performance in task 2, which incorporated a set of stimuli varying in virtual grid spatial frequency but not in electrical amplitude cues, was still above chance level. This could be explained by the presence of the already mentioned positional cues- the non-rewarded grid had a higher spatial frequency and was thus present only on the starting phase of a whisker protraction. To test if the animals based their behavioral reports on knowledge of the position of their whisker in space a set of non-rewarded virtual grids was introduced in task 3. The starting points of these virtual grids were spaced equally over the region of whisker movement, with the two outmost lines of the rewarded grid coinciding with the first line of the most caudal non-rewarded grid and the last line of the most rostral non-rewarded grid respectively. This was the experimental condition in which all animals reached their lowest discrimination indices. The non-rewarded virtual grids were not correctly rejected when delivered via electrical stimulation matching the electrical stimulation for a rewarded virtual grid. In this task the information about the position of the grids was still available to the animals but was probably not as consistent as compared to task 2, hence the failure of the animals to discriminate.

The Go/No-Go paradigm as used in this thesis work was behaviorally challenging for the experimental animal because it demanded from the animal to give a report about the identity and not the mere presence of a stimulus. It could well be that the rats could detect the absolute position of a stimulus with respect to the snout but still failed to discriminate when given only spatial frequency cues. The fact that the performance on the Go/No-Go discrimination task as presented here was at its best when there was an electrical amplitude cue together with the spatial frequency cue of the virtual grids suggest a relative unimportance of frequency cues during active discrimination and speaks for the use of instantaneous kinematic fast intensity cues, such as mimicked by the electrical pulse amplitude. In the real world of a rat such instantaneous cues are present as kinematic events observed in the whisker trajectory during real texture exploration (Wolfe et al., 2008). The number and absolute size of these so called slip-stick events, which are high-velocity and high- acceleration changes in the whisker trajectory, corresponds to the coarseness of the sampled texture and is potentially used by the animal for texture discrimination. The transfer function of the activation of receptors in the whisker follicle and the mechanical vibrations of the whisker shaft during these slip-stick events to the sensory cortex is still not known. In their work, Waiblinger et al. investigated if the instantaneous characteristics of the vibrotactile signal can be used for perception (Waiblinger et al., 2013). The authors trained rats on a detection of change task in which constant pulsatile vibrotactile stimulation was delivered near the base of one whisker of a head fixed rat with a piezo element.

In a first test the animals were asked to detect the occurrence of a second stimulus which had either the same overall intensity or the same frequency as the comparison stimulus. In both of these cases the test stimulus provided an absolute amplitude difference cue to the animals and they were able to discriminate. In a second step, the kinematics of both stimuli was matched, so that there was no change in amplitude, velocity or acceleration and here the animals were no more able to discriminate.

With the experimental set-up used in my work it was not possible to show that rats can reliably discriminate between stimuli with different spatial frequencies. The experimental results found by Waiblinger (Waiblinger et al., 2013) and the ones described by my work argue in favor of a similar coding scheme for passive and active perception. Out of the three main parameters relevant for the sensory percept described before- the frequency, the intensity and the kinematic cues- both studies found the instantaneous kinematics to be of the highest importance for the sensory system. In the electrical stimulation experiments the kinematic cue is mimicked by the amplitude of the electrical pulse, where as the analog of the mechanical intensity cue, would be the integrated electrical current delivered per virtual grid.

Another finding of the present study is that none of the experimental animals noticeably modified the parameters of its whisking strategy when working on the different discrimination tasks. The velocities with which the rats crossed the rewarded virtual spatial grids were only minimally diverging from the velocities used to cross a non-rewarded grid. Moreover, there were very few instances in which a change in the velocity of the whisker could be correlated with the decision of the animal on correct versus wrong trial both for the rewarded and the non-rewarded stimuli. This difference could hence be disregarded as biologically insignificant. The spatial frequency differences between the rewarded and non-rewarded grids were not fully utilized by the animals- the behavioral performance of all three rats was at its worst when the stimuli were devoid of electrical amplitude cues (task3).

From preliminary data and the experiments presented in this thesis, as well as based on the findings of Waiblinger et al. (2013), the instantaneous kinematic events are to be considered the most probable candidate feature of a surface which is used for discrimination.

A valid consideration for future experiments is to use the highly controllable electrical stimulation to mimic the stick and slip events and to construct virtual textures resembling real textures and present them to the animal. To arrive at such a strictly controlled way to deliver the electrical stimulation, one should first investigate the physical parameters changing when a whisker touches real objects during active whisking. The vibrations occurring in a whisker shaft upon object touch most probably carry the important sensory information used by the animal. It would then be of interest to investigate how shaft vibrations relate to instantaneous kinematic events such as the slip-stick events and to action potential generation in the trigeminal ganglion, the first stage of sensory processing. By recording the movements of an identified whisker rubbing along a well defined surface at certain distances by using videography one can estimate the forces acting on the whisker shaft at specific positions and how they depend on the whisker velocity and surface roughness. Such highly precise surfaces have been described and used in human finger tip research (Skedung et al., 2013). Since the instantaneous kinematic events are the major candidate feature for discrimination, one should investigate how their properties (their kinematics and probability of occurrence) change under different conditions such as different texture roughness or different whisker velocities. Then in a second step one can use probabilistic electrical stimulation with a certain amplitude distribution to mimic these different slips. This will allow us to go from investigation of the frequency parameter as described in this thesis, to the investigation of kinematic events such as the electrical pulse amplitude during active whisking. For example, the probability of occurrence and the amplitude of the electrical stimuli can be made dependent on the whisking speed to see if the animals would adopt a specific strategy to optimize their percept. The use of electrical stimulation to construct virtual textures is artificial but it will allow for the highest degree of stimulus control and will allow us to connect the movement strategies and performance of the animals to the occurrence of kinematic cues. The difficulty of the task could also be reduced by eliminating the now existing memory component and replacing the Go/No-Go task with a different behavioral paradigm. Two possibilities would be a detection of change task in which the stimuli to be discriminated come seamlessly one after the other, or a Yes/No task which has a symmetric reward matrix and thus keeps the motivation of the animal to work on the task high.

Even at this preliminary stage of investigation, the outcome of this project opposes current views on the functioning of the somatosensory system of primates. It is, in my view, unwise to disregard the inability of rats to faithfully discriminate spatial frequencies as a simple drawback of the rodent somatosensory system. Even though rodents and primates are distinctly different species, separated by evolution, it is not impossible that their somatosensory systems hold common processing mechanisms. It would thus be extremely interesting to see how a non-human primate would fare on an active perception spatial frequency discrimination task as the one described in this work, which provides only frequency differences and no other sensory cues.

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7. Supplementary

Statistical significances found by an independent samples Kruskal-Wallis test for pairs of the behavioral responses of all animals and each task.

Table 1 Bonferroni- adjusted significance values for the medians of the grid cross number distributions in respect to the behavioral response of all animals for task 1 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	CR-hit	0.001
	FA-hit	0.0001
2	CR-hit	0.0001
	FA-hit	0.0001
	CR-FA	0.0001
	CR-miss	0.0001
	miss-hit	0.041
3	CR-hit	0.0001
	CR-FA	0.0001

Table 2 Bonferroni- adjusted significance values for the medians of the whisker velocity distributions in respect to the behavioral response of all animals for task 1 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	CR-hit	0.005
2	CR-hit	0.0001
	FA-hit	0.009
3	CR-hit	0.004

Table 3 Bonferroni- adjusted significance values for the medians of the grid cross number distributions in respect to the behavioral response of all animals for task 2 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	CR-hit1	<0.001
	CR-hit2	<0.001
	CR-hit3	<0.001
	CR-hit4	<0.001
	FA-hit1	<0.001
	FA-hit2	<0.001
	FA-hit3	<0.001
	FA-hit4	<0.001
2	CR-hit1	<0.001
	CR-hit2	<0.001
	CR-hit3	<0.001

	CR-hit4	<0.001
	CR-miss1	0.017
	CR-FA	0.049
	hit2-miss2	0.036
	FA-hit1	<0.001
	FA-hit2	<0.001
	FA-hit3	<0.001
	FA-hit4	0.001
3	CR-hit1	0.009
	CR-hit2	<0.001
	CR-hit3	<0.001
	CR-hit4	<0.001
	CR-miss1	0.001
	hit1-hit2	<0.001
	hit1-hit3	<0.001
	hit1-hit4	<0.001
	FA-hit1	<0.001
	FA-hit2	<0.001
	FA-hit3	<0.001
	FA-hit4	<0.001

Table 4 Bonferroni- adjusted significance values for the medians of the whisker velocity distributions in respect to the behavioral response of all animals for task 2 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	CR-hit1	<0.001
	CR-hit2	<0.001
	CR-hit3	<0.001
	CR-hit4	0.006
	FA-hit1	<0.001
2	No significant differences between samples	
3	CR-hit1	<0.001
	hit1-hit2	<0.001
	hit1-hit3	<0.001
	hit1-hit4	<0.001
	FA-hit1	<0.001

Table 5 Bonferroni- adjusted significance values for the medians of the grid cross number distributions in respect to the behavioral response of all animals for task 3 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	hit-CR2	0.001
	hit-CR3	0.001
	miss-CR2	<0.001
	miss-CR3	<0.001

	CR2-FA2	0.046
2	hit-CR1	<0.001
	hit-CR2	<0.001
	hit-CR3	0.009
	miss-hit	0.001
	hit-FA1	<0.001
	hit-FA2	<0.001
	hit-FA3	<0.001
3	hit-CR1	<0.001
	hit-FA1	<0.001
	hit-FA2	<0.001

Table 6 Bonferroni- adjusted significance values for the medians of the whisker velocity distributions in respect to the behavioral response of all animals for task 3 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	No significant differences between samples	
2	hit-CR2	0.001
	hit-CR4	0.009
	hit-miss	0.011
	hit-FA3	0.033
	hit-FA4	<0.001
3	hit-CR1	0.026
	hit-FA1	0.003
	CR3-FA3	0.034

Two of the animals did not reach proper levels of discrimination in task 4, which used the same set of stimuli as task3 but providing additional electrical pulse amplitude cue for the non-rewarded stimuli. For these I found that one animal crossed the rewarded grid more often than the non-rewarded grids (in median 8 times for a rewarded grid and 1 to 10 times for a non-rewarded grid) and one animal chose to cross all stimuli equally often (in median 4 times for a rewarded grid and 3 times for a non-rewarded grid) (Fig.1 A). I found very few correlations between the velocity with which a whisker crossed a virtual grid and the behavioral response of the animal (Fig. 1 B). In one animal the most rostral of the non-rewarded grids was crossed with median velocities slightly lower than the median velocities used for a rewarded grid (474 versus 527 mm/s respectively, Kruskal-Wallis non parametric test p=0.006).

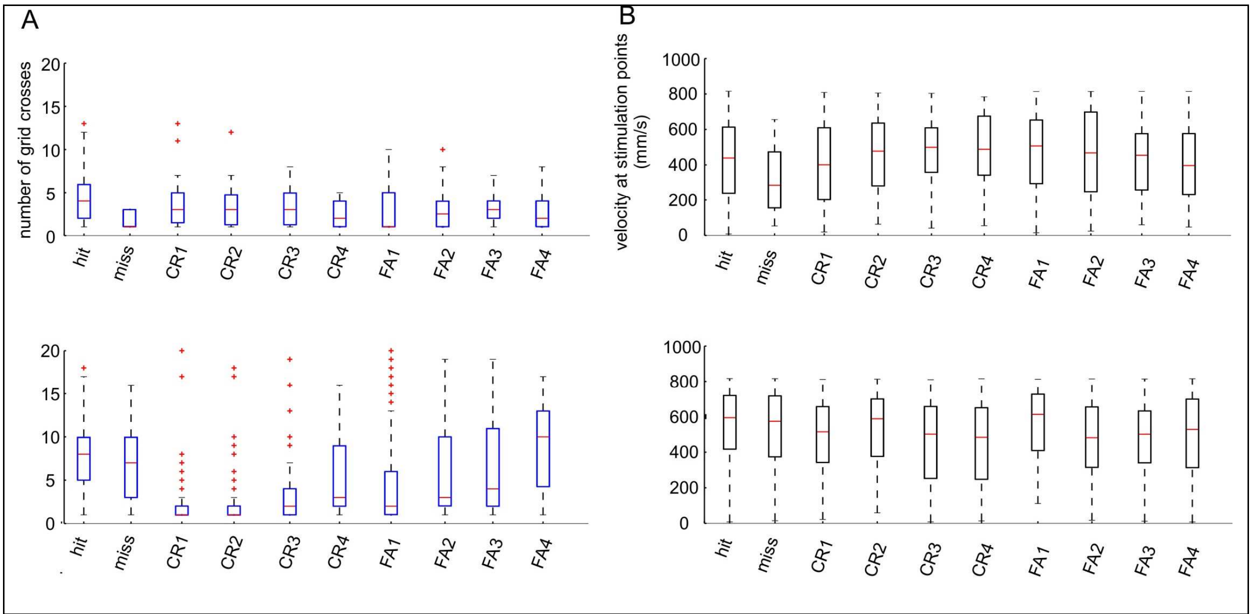

Figure 1 A: Box-whisker plots of the number of grid crosses during all sessions of task 4 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses.

B: Box-whisker plots of the velocities with which the whisker crossed the stimulation points of the grid during all sessions of task 4 with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses

Table 7 Bonferroni- adjusted significance values for the medians of the grid cross number distributions in respect to the behavioral response of all animals for task 4 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	hit- CR4	0.031
	hit-FA1	0.023
2	hit-CR1	<0.001
	hit-CR2	<0.001
	hit-CR3	<0.001
	hit-CR4	0.003
	hit-FA1	<0.001
	hit-FA2	<0.001

	hit-FA3	0.002
	CR1-FA1	0.004
	CR2-FA2	<0.001
	CR3-FA3	<0.001
	CR4-FA4	<0.001
3	hit-CR1	<0.001
	hit-CR2	<0.001
	hit-CR3	<0.001
	hit-miss	0.027
	hit-FA1	<0.001
	hit-FA2	<0.001
	hit-FA3	<0.001

Table 8 Bonferroni- adjusted significance values for the medians of the whisker velocity distributions in respect to the behavioral response of all animals for task 4 (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	miss-FA1	0.038
	miss-FA2	0.025
	miss-CR3	0.012
	miss-CR4	0.025
2	hit-CR3	<0.001
	hit-CR4	<0.001
	hit-FA2	<0.001
	hit-FA3	<0.001
	hit-FA4	<0.001
	miss-CR3	0.032
	miss-CR4	0.001
	miss-FA2	0.039
	miss-FA3	0.007
3	hit-CR4	0.006
	miss-CR1	0.008
	miss-CR2	0.009
	miss-FA2	0.006
	hit-miss	0.020

For two of the animals I repeated test 1 and found the opposite behavioral strategies. The first animal chose to cross the rewarded grid more often than the non-rewarded (in median 6 times for a hit versus 1 time for a correct rejection), whereas the second animal crossed the rewarded grid in median 3 times for a hit and the non-rewarded grid 7 times for a correct rejection (Fig. 2 A).

The median velocities in the first animal were higher for a hit (574mm/s) than for a correct rejection (506mm/s, Kruskal-Wallis non-parametric test, p<0.001) or false alarm (485mm/s, Kruskal-Wallis non-parametric test, p<0.001) (Fig.2 B). For the second animal I found a statistical difference between the median velocities used in a hit versus false alarm case (510 mm/s for a hit and 440 mm/s for a false alarm response, Kruskal-Wallis non-parametric test, p=0.001), but no statistical difference in a hit versus correct rejection comparison (510mm/s for a hit and 480 mm/s for a correct rejection, Fig 16 A). Again, there was no statistical difference in the whisker velocity distributions for correct rejections versus false alarms.

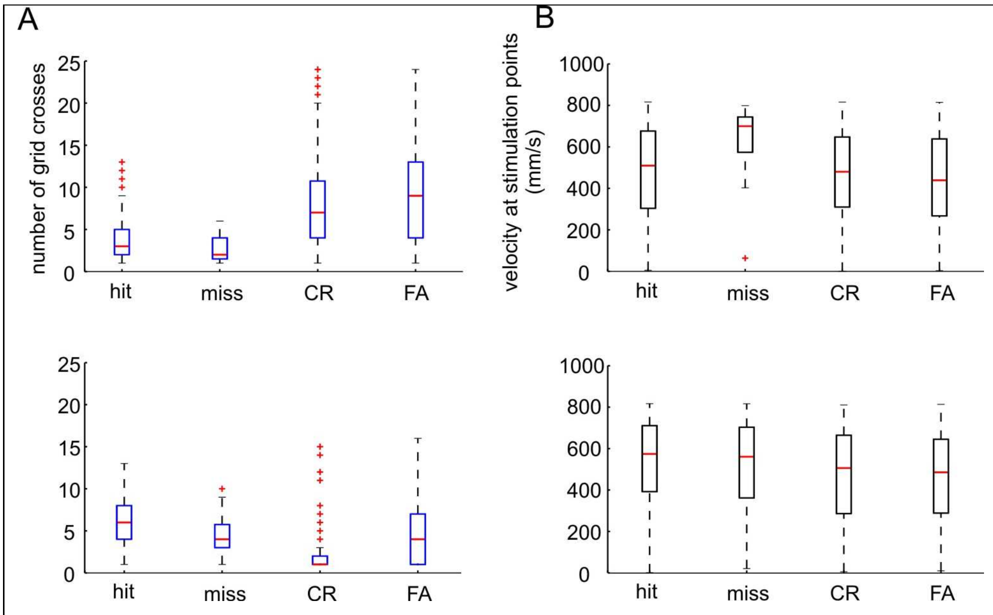

Figure 2 A: Box-whisker plots of the number of grid crosses during all sessions of the repetition of task 1 (control) with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses

B: Box-whisker plots of the velocities with which the whisker crossed the stimulation points of the grid during all sessions of the repetition of task 1(control) with respect to the identity of the grid and the response of the animal. For each box plot the upper and lower boundary of the box plot mark the 25th and 75th percentile of the distribution, the central red line is the median of the distribution, the broken line (whisker) extends to the most extreme data points of the distribution and the outliers are plotted separately with red crosses.

Table 9 Bonferroni- adjusted significance values for the medians of the grid cross number distributions in respect to the behavioral response of all animals for task 1 (control) (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	hit-CR	<0.001
	hit-FA	<0.001
	miss-FA	0.031
2	Hit-CR	<0.001
	Hit-FA	<0.001
	Miss-CR	<0.001
	CR-FA	<0.001

Table 10 Bonferroni- adjusted significance values for the medians of the whisker velocity distributions in respect to the behavioral response of all animals for task 1 (control) (Independent samples Kruskal-Wallis Test)

animal	Sample1- Sample2	Bonferroni- adjusted p-value
1	hit-FA	0.001
	miss-FA	0.044
2	hit-CR	<0.001
	hit-FA	<0.001