Visual perception of artificial and nature-inspired stimuli in an open-source python-based touchscreen chamber for operant conditioning

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der RWTH Aachen University zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigte Dissertation

vorgelegt von

Christopher Wiesbrock, M.Sc.
aus
Bielefeld, Deutschland

Berichter: Universitätsprofessor Dr. Björn Kampa

Universitätsprofessorin Dr. rer. nat. Geraldine Zimmer-Bensch

Tag der mündlichen Prüfung: 05.11.2020

“The important thing is to not stop questioning. Curiosity has its own reason for existing.”

- Albert Einstein

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Contributions

The touchscreen chamber training was a part of the bachelor thesis “Touchscreen based orientation discrimination and oblique effect in mice” by Stephanie Anna Eichhorn. She assisted with the training of the animals under my supervision.

The staircase procedure without retraining was a part of a research internship by Jenice Linde. She assisted with animal training and data recordings under my supervision.

The spatial frequency discrimination task in chapter 3 was a part of the master thesis “Spatial frequency discrimination of nature-inspired stimuli in mice and humans” by Jenice Linde. She created the stimuli and assisted with the animal training and data recordings under my supervision. The widefield imaging in Chapter 3 was recorded and analyzed by Gerion Nabbefeld.

Any experiment was designed by Björn Kampa and myself. Chapters 2 and 3 are planned to be published together by Christopher Wiesbrock, Stephanie Eichhorn, Jenice Linde and Björn Kampa. Parts of chapter 4 are planned to be published by Christopher Wiesbrock, Elisabeta Balla, Jenice Linde and Björn Kampa. Chapter 5 is planned to be published by Christopher Wiesbrock and Björn Kampa.

Acknowledgments

First and foremost I want to thank Professor Dr. Björn M. Kampa for the opportunity to work on this thesis in his lab. In the past years, I learned a lot about Neurophysiology, but I also learned a lot beyond the scientific path. Dear Björn, I think the last few years were the most interesting journey of my professional life. You gave me the space to follow my own scientific ideas and also to make my own mistakes, from which I learned the most. I really appreciate all the opportunities you gave me. Thank you.

I also would like to thank Professor Dr. Geraldine Zimmer-Bench for being the second supervisor of this thesis.

Jenice Linde, I also would like to thank you for letting me supervise you, for your master thesis and in a research project. I think you brought a lot of drive back to the Motion Cloud-story. Thank you as well for proof-reading and the helpful remarks.

Stephanie Eichhorn, my first bachelor student, you helped me a lot with all the troubleshooting on the touchscreen chamber. You were absolutely essential to establish the touchscreen chamber as a stable method in the lab.

Carsten Seeliger, we spent a lot of time together, but I think in the end it was absolutely worth it. Your ideas on the optomotor box and your critical thinking were a perfect base for all the exciting upcoming research which will be done in this setup.

Julia Büscher, Anna Pierling, Jaqueline Rudolf, Fabio El Yassouri, you all took part in one of my projects within a research project. It was always nice to have the capacity to try out new things and most of it gave meaningful hints for upcoming projects.

All members of the Kampa Lab, it was a pleasure to work with you. But I do not only appreciate the lively discussions with Magda about "what could go wrong" or the technical discussions with Gerion, but I also loved to talk about motor-racing and deep learning algorithms with Thomas. I learned a lot from Alex, who started earlier with behavioral experiments and had some needful hints. I also want to thank Natalja and Kerstin for being the nicest office mates, one could have. And I would like to thank Claas

for his edgy jokes and outstanding cakes. Also a big thank you to the Spider Lab, who gave me a new shelter for my touchscreen chamber.

Thanks to Michael for keeping our computers running. Thanks to Sylvia for administrative support. Thanks to Sandra for the maintenance of the animal facility. Thanks to the guys from the Biology Workshop, who realized all my weird ideas.

Thanks to Elisabetta who helped me a lot in the final phase of my thesis and taking over the Motion Cloud project. I am sure, there is a lot more you will discover.

Thanks to Laurent Perrinet for so much input on the Motion Clouds, thanks to Spencer L. Smith for crucial hints for building the touchscreen chamber.

Last, but not least, I would like to thank my kids Joel and Amicia, for all their emotional support. One day you will realize how important you were for this piece of paper. Chantal, my wife, who supported me from the first idea of studying biology until today (and hopefully further on). This thesis would have never happened without you. I would also like to thank my family. You all made this happen, by keeping me motivated, when I lost motivation.

Abstract

The visual system of the mouse is a long-standing component of neuroscientific research. There is an ongoing discussion about how to stimulate the visual system in a meaningful way. Here, stimulation competes with artificial and nature-inspired stimuli. The main difference is the different degree of parameterization of the two stimulus approaches. The more clearly the parameters are defined, the more likely it is that the image statistics will be associated with an experimental result. With the help of so-called motion clouds, a stimulus has been created that is able to reproduce natural image statistics while at the same time providing a high degree of parameterization.

In the present thesis the motion clouds will be tested in a behavioral experiment with mice with regard to the influence of individual parameters. First, a test setup was developed based on the principle of operant conditioning. This system, the so-called touch screen chamber, is a highly automated tool for animal training. At the same time, the modular and flexible hardware and software combination offers the possibility to work with stimuli that can be freely selected.

A first test of the experimental setup was carried out by experimentally verifying a test of the effect of over-representation of neurons in the primary visual cortex that respond to horizontal and vertical stimuli. This circumstance is also related to the orientations in natural photographs, which on average show an overrepresentation of these orientations. In this thesis it could be shown that this effect in mice leads to a better discrimination of orientations when they are horizontal or vertical than when they are in between. However, this effect could be eliminated in the experiment by training the animals to distinguish the underrepresented orientations from others.

In the next step the experimental concept was applied to the nature-inspired motion clouds. It is assumed that the processing in the cortex is based on parallel connected sensory channels. Here, two experiments are presented in which the animals learn to distinguish between two differently oriented motion clouds, and the differentiation of motion clouds with a different spatial frequency. It can be shown that different bandwidths of the spatial frequency, as it occurs in nature, do not influence the orientation difference. In contrast, a higher bandwidth of orientations in the stimulus leads to an improved discrimination of the spatial frequency. This supports the hypothesis that several activated sensory channels improve experimental performance. Finally, the experiments, in particular the training program and the orientation discrimination for the education of biology students were modified and proved to be a sound educational concept

Zusammenfassung

Das visuelle System der Maus ist ein langjähriger Bestandteil neurowissenschaftlicher Forschung. Eine anhaltende Diskussion wird darüber geführt, wie man das visuelle System sinnvoll stimuliert. Hierbei konkurrieren die Stimulation mit künstlichen und natur inspirierten Stimuli. Der Hauptunterschied liegt im unterschiedlichen Grad der Parametrisierung der beiden Stimulus-Ansätze. Je klarer die Parameter definiert sind, desto eher sind die Bildstatistiken in Verbindung mit einem experimentellen Ergebnis zu verbinden. Mit Hilfe sogenannter Motion Clouds wurde ein Stimulus geschaffen, welcher sowohl natürliche Bildstatistiken abbilden kann, aber gleichzeitig auch ein hohes Maß an Parametrisierung mitbringt.

In der vorliegenden Thesis sollen die Motion Clouds in einem Verhaltensversuch mit Mäusen mit Blick auf den Einfluss einzelner Parameter getestet werden. Hierbei wurde zunächst ein Versuchssetup entwickelt, basierend auf dem Prinzip der operanten Konditionierung. Dieses System, die sogenannte Touchscreen Chamber, ist ein hoch automatisiertes Werkzeug für das Tiertraining. Gleichzeitig bieten die modularen und flexiblen Hardware- und Softwarezusammenstellung die Möglichkeit auch mit Stimuli zu arbeiten, die frei wählbar sind.

Eine erste Prüfung des Versuchsaufbaus erfolgte anhand der experimentellen Überprüfung eines Tests der Auswirkung einer Überrepräsentation von Neuronen im primären visuellen Kortex, welche auf horizontale und vertikale Reize reagieren. Dieser Umstand steht auch in Verbindung mit den Orientierungen in natürlichen Fotoaufnahmen, welche im Schnitt eine Überrepräsentation eben dieser Orientierungen aufweisen. Es konnte in dieser Thesis gezeigt werden, dass dieser Effekt in Mäusen dazu führt, dass Orientierungen besser unterschieden werden können, wenn diese horizontal oder vertikal sind, als wenn sie dazwischen liegen. Dieser Effekt ließ sich im Experiment allerdings auch aufheben, indem die Tiere gezielt darauf trainiert wurden die unterrepräsentierten Orientierungen von anderen zu unterscheiden.

Im nächsten Schritt wurde das Versuchskonzept auf die natur-inspirierten Motion Clouds verwendet. Es wird hierbei angenommen, dass die Verarbeitung im Kortex auf der Basis

parallel geschalteter sensorischer Kanäle basiert. Hierbei werden zwei Versuche vorgestellt in denen die Tiere lernen, zwei unterschiedlich orientierte Motion Clouds zu unterscheiden, sowie die Differenzierung von Motion Clouds mit einer unterschiedlichen räumlichen Frequenz. Hierbei kann gezeigt werden, dass unterschiedliche Bandbreiten der räumlichen Frequenz, wie sie auch in der Natur vorkommt, keinen Einfluss auf die Orientierungsunterschiedung hat. Im Gegensatz dazu führt eine höhere Bandbreite an Orientierungen im Stimulus zu einer verbesserten Unterscheidungsfähigkeit der räumlichen Frequenz. Dies unterstützt die Hypothese, dass mehrere aktivierte sensorische Kanäle eine Verbesserung der Versuchsleistung erzielen. Abschließend wurden die Versuche, insbesondere das Trainingsprogramm und die Orientierungsunterscheidung zur Ausbildung von Biologie-Studenten modifiziert und erwies sich als fundiertes Ausbildungskonzept.

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List of Abbreviations

2AFC - Two alternatives forced-choice
cm - centimeter
cpd - cycles per degree
csv - comma separated value
d - days
d’ - Discriminability index
Eq - Equation
Fig - Figure
fmRI - functional magnetic resonance imaging
g - gramm
gb - Gigabyte
GHz - Gigahertz
GUI - graphical user interface
h - hours
IR - infrared
ISI - Interstimulus interval
ITI - Intertrial interval
LED - light-emitting diode
LGN - lateral geniculate nucleus
Log - logarithmic
LSD - Least significant difference
ml - milliliter
mm - millimeter
min - minutes
n.s. - not significant
OKR - optokinetic response
OMR - optomotor response
PAL - paired-associated learning
PVC - polyvinyl chloride
S - seconds
SC - superior colliculus
SD - standard deviation

SEM	-	standard error of the mean
SSIM	-	structural similarity
Tab	-	table
USD	-	US-dollar
V1	-	Primary visual cortex

Chapter 1: General Introduction

A walk in the forest on a beautiful spring morning gives us a large number of visual impressions. One can see trees, animals, flowers and maybe a river as well. Our visual system derives from this input several pieces of information. If we see a squirrel walking at us, we know without touching or smelling the animal, that there is a squirrel and not a flower. On the first look, this seems to be rather trivial, but on the level of cortical integration, we face a problem that is described for decades of research. In fact, the squirrel gives us a lot of information just from its presence. We can see a tiny brownish colored moving structure represented on our retina. From our memory, we can classify the squirrel as a squirrel, but which structures give us the capability to do so?

Breaking it down to the visual information, we see the edges, between the squirrel and the environment. In between the edges, we see the color of the fur and we see the anatomical characteristics of the squirrel. Some of this information is also decoded in the primary visual cortex. Edges are represented as orientations, anatomical characteristics like the relation between the head, the body and the limbs can be interpreted as spatial information. This gives us already a big hint, that there is a small rodent instead of a large tree. In opposite to that, a large number of studies in the field of visual research were focused on the use of artificially created stimuli, like simple bars or gratings.

There are comprehensible reasons arguing for the use of artificial stimuli. On the one hand, artificial stimuli are parameterized to a maximal extent. From the mathematical point of view, this makes the formalization of visual perception into a model more robust (Rust & Movshon 2005). Contradicting the idea of this way to get a deeper insight into visual perception is the fact that most models fail when they are tested with naturalistic images, like photographs. On the other hand, there was also no robust model based on experiments with natural images to give a comprehensive insight into the perception of natural scenery. Olshausen (2003) made the statement, that there is still the lack of a theoretical framework to create experiments that are exactly addressing the question for the visual perception of natural images. Hereby, the author identified one of the key problems based on missing knowledge in terms of pattern analysis. This argument comes with several recent problems due to the question of the exact computation in the primary visual cortex.

All in all, it can be concluded that a simplistic approach with artificial stimuli needs a completely different framework than investigations of the functional processing of naturalistic stimuli. As Rust & Movshon proposed, to investigate the perception of naturalistic stimuli, it needs a different mindset in terms of the basic model.

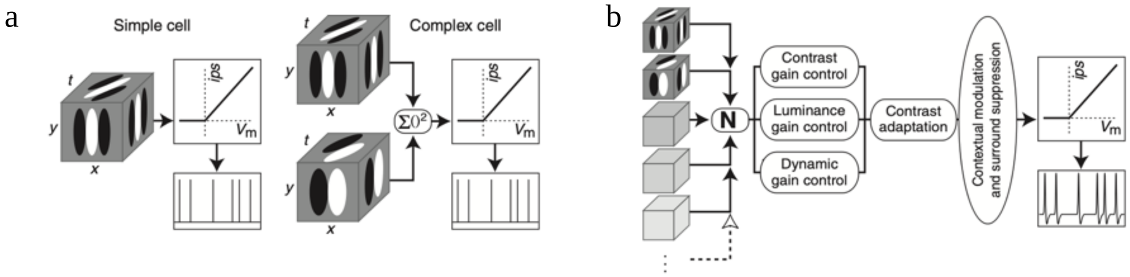


Figure 1.1: Models to derive cortical cell activity depending on the stimulus (from Rust & Movshon 2005)

a) The simple cell model shows that this cell type responds with activity to a certain stimulus. On the x and y-axis of the cube, the spatial information of the stimulus is shown. The t-axis shows the change over time. A complex cell reacts to more than one stimulus characteristic. If a stimulus shows different spatial frequencies, they are summed and evoke activity by a complex cell.

b) The model for naturalistic stimuli adds more factors. Image N consists of several individual characteristics like different spatial frequencies or different orientations. Mechanisms, which are added are contrast gain control, luminance gain control and dynamic control, which results in contrast adaptation. Another factor added is contextual modulation and surround suppression which then results in a neuronal response

The goal of this thesis is not to test the models shown in Figure 1.1, but to use a distinct approach. While Rust & Movshon (2005) and Olshausen (2003) mainly focused on the question of how the activity of neuronal ensembles can be explained, we chose a different approach. This thesis focuses on the idea to test the differences between artificial and natural-inspired stimuli with aiming for the functional output of the stimulation with certain stimuli.

One way to measure the functional output of such a complex phenomenon as the perception of naturalistic images is to apply behavioral experiments. Mice as a model organism have a long tradition in the research of the visual system. To apply the experiments, our first goal was to find a way to test the animals' visual system in a way that restricts their natural behavior as little as possible. Another challenge was to provide an experimental approach that is universally usable. To our belief, it is more than important to collaborate on a big question like the perception of natural stimuli, with most other researchers as possible. Therefore, we propose an open-source python-based touchscreen chamber for operant conditioning (Chapter 2). In this operant conditioning chamber, we were able to test different artificial stimuli with respect to questions which arose from the perception of natural sceneries. The analysis of naturalistic images with view on the edge orientations contained in these images revealed a so-called oblique effect. This means, that there is an overrepresentation of horizontally and vertically orientated edges in natural sceneries (Girshick et al. 2011). This is represented in the tuning of cortical neurons in the primary visual cortex as well. In a visual discrimination task, we tested the functional consequence of this overrepresentation with respect to the learning-history of mice (Chapter 3).

After we established a solid framework, we were able to include nature-inspired stimuli. As a disadvantage of naturalistic images, it has to be mentioned, that they do not provide the level of parameterization and standardization as artificial stimuli do. A proper approach, compared to photographs or videos from natural sceneries, are so-called motion clouds. They can be characterized as hybrid stimuli, using basic principles from artificial stimuli, but representing naturalistic image statistics at the same time (Chapter 4).

1.1. The visual system

The visual system computes visual signals as a part of the central nervous system. The information is given by photons, which are first recognized by the retina. In the retina, specific light-sensitive cells, called rods and cones detect the incoming photons. Rods are sensitive to light and enable us to perceive different levels of brightness. Cones are sensitive to light of specific wavelengths, which makes them important for the perception of color. Humans, as a trichromatic species, have three different types of cones to detect different wavelengths of light, which results in the perception of different colors (Oyster 1999). The main structure of the retina is characterized by two distinct layers: *Stratum pigmentosum* and *Stratum nervosum*. The *Stratum pigmentosum* connects the retina with the choroid, which protects the retina and delivers nutrients to maintain the physiological function. Another function of the *Stratum pigmentosum* is to assimilate metabolic products from the retinal cells. The sensory cells are located in the *Stratum nervosum*. Rods and cones project to bipolar cells, horizontal cells and amacrine cells, which are interconnected and are having the function of retinal interneurons. The ganglion cells' axons build up the optic nerve, which transmits the visual information into the brain in the form of action potentials. The retina is also the place, where the first tuning processes are happening in terms of orientation selective ganglion cells (Levick 1967). Via the *chiasma opticum* the retinal input proceeds mainly to the contralateral hemisphere of the brain. The *lateral geniculate nucleus* (LGN) is the gateway to the cortex and further modulates the signal before it arrives there (O'Connor et al. 2002, Sylvester et al. 2005). Therefore, the LGN is described as a selective filter (Burr et al. 1994). Nevertheless, the LGN also receives input from the primary visual cortex and is a first-order relay center for attention. Dorsally of the LGN within the thalamus, the pulvinar is a second relay center for the modulation of attention (Robinson & Peterson 1992). It receives input from several brain areas and the *superior colliculus*. The latter mentioned is an important structure for the modulation of reflexive eye movement. As part of the mesencephalon, the superior colliculus is located in the most upper layer of the midbrain, the so-called tectum. The optic radiations are the projection from the LGN to the primary visual cortex (occipital lobe), and therefore the first point of cortical sensory processing (Figure 1.2). The visual pathway is organized with different receptive fields, which apply a tuning on different hierarchical stages as well as in the different layers of V1 (Niell & Stryker 2008). From retina to cortex, the receptive fields are increased in size (Coogan & Burkhalter 1993, Wang & Burkhalter 2007, Wang et al. 2011). This is important, because it applies a preprocessing before information arrives at the cortex, but the biggest receptive fields are in the cortex, so we assume the most relevant processing steps there, due to a higher optical density (Wang et al. 2011).

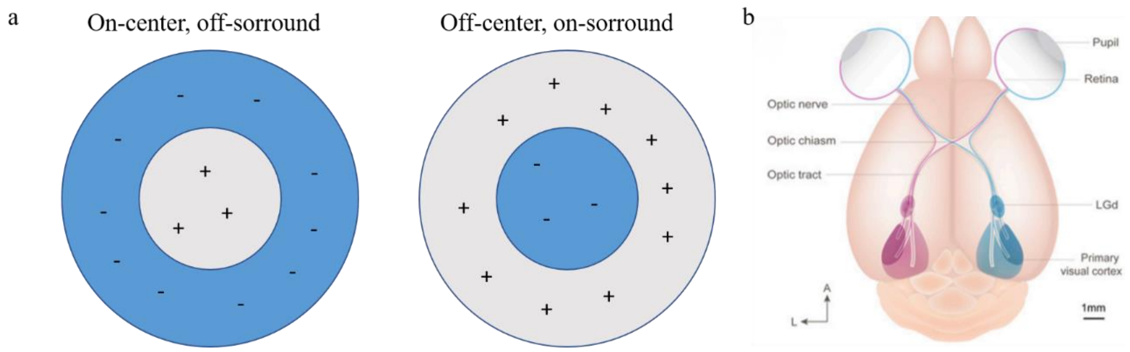


Figure 1.2: Schematic overview of receptive fields and the visual pathway in mice (Hawrylycz et al 2016).

a) Schematic view on receptive fields. On-center, off-sorround receptive fields have excitatory cells in the center, while surrounding cells are silenced during activity. Off-center, on-sorround receptive fields are vice-versa

b) The figure shows the schematic overview of the mouse visual system starting from the pupil and the retina. The signals are transferred to the primary visual cortex and the LGN via the optic nerve and the optic chiasm.

1.2. The perception of artificial and natural stimuli

One of the leading questions of this thesis is the perception of artificial stimuli, especially sine-wave gratings. Besides that, there is also the question, how natural stimuli are perceived.

In a comprehensive study, Roth et al. 2012 tested the neuronal responses of gratings, with different orientations, spatial frequencies and temporal frequencies. In conclusion, they proposed a model, including the functional characteristic of the primary visual cortex and the posteriormedial cortex in mice.

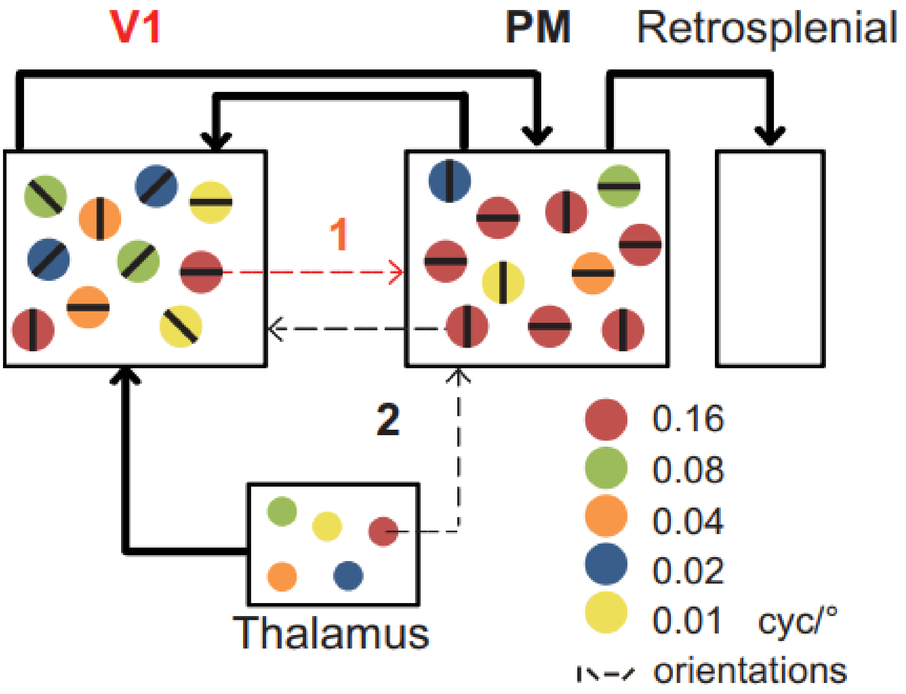


Figure 1.3: Possible computation pathways for orientations and spatial frequencies of the visual system (Roth et al. 2012*)* *Orientations and spatial frequencies are processed in the primary visual cortex and the posteriomedial area. There are connections as well from the thalamus to V1, it is not known if there are also connections to PM. There are connections in both directions between V1 and PM. The role of the retrosplenial cortex is not known.*

This model shows different channels in these two structures, tuned for different orientations and spatial frequencies. This leads to the assumption, that the perceptual system is organized in distinct sensory channels. Figure 1.3 illustrates the orientation and spatial frequency tuning in V1 and PM. The functional role of retrosplenial cortex is unknown.

The study by Roth et al. 2012 showed in mice that neurons tuned to cardinal orientations, that is vertical and horizontal, are highly overrepresented in V1 and even more in PM. Similar results have also been shown in ferrets, cats or primates. Interestingly, it could also be shown in humans that orientation discrimination is better around cardinals compared to obliques suggesting an improved perception driven by the higher abundance of processing orientation tuned neurons (Girshick et al. 2011).

The abundance of sensory channels, e.g. more neurons are tuned for cardinal orientations, is also reflected in natural image statistics (Girshick et al. 2011). Following this hypothesis, a more enriched stimulus, including several orientations and spatial frequencies, should activate several sensory channels. A study in anesthetized ferrets, using natural photographs, showed that the primary visual cortex represents the natural image statistics in terms of orientation and spatial frequency (Smyth et al. 2003). This was measured via single cell electrophysiological recordings. From the recordings, a receptive field map was derived. A fourier transformation spectral analysis showed then the tuning of the measured cells. Another study about the perception of natural textures in primates showed that primary visual cortex processes orientations whereas the correlation of these orientations in texture patterns are processed in higher visual areas, mainly in V2 (Freeman et al. 2013). In mice this computation of higher order statistics in visual stimuli might, however, already occur on the level of the primary visual cortex (Muir et al. 2015, Muir et al. 2017). The addition of bandwidth to a stimulus led in the past to different results. A speed discrimination task in humans showed that a larger spatial frequency bandwidth did not give a functional advantage (Simoncini et al. 2012). The role of stimulus bandwidths is a main focus of this thesis. In a discrimination task for natural sceneries in mice, Yu et al. 2018 identified the structural similarity index of the applied natural images as the variable to determine the discrimination difficulty. This was achieved by a gaussian filter, which reduced the high spatial frequencies content of the image. The dominant orientation information, especially the horizon in the photograph stayed intact. For mice the dominant feature in natural images was the broad difference between a bright sky and a dark ground separated by the horizon. However, this study could not further elaborate how the orientation and spatial frequency content of natural images play a role in visual perception. This leads to several questions, addressed in this thesis. What is the functional consequence of the unbalanced representation of orientations in the primary visual cortex and other areas? Which functional consequences can be derived from the stimulation with enriched stimuli, including a bandwidth of orientations and spatial frequencies? Is the additional information, and therefore more activated sensory channels, in any way advantageous for decision making? These questions will be tested in terms of behavioral experiments in mice.

1.3. Mice as model organisms

The groundbreaking findings by Hubel & Wiesel (1959) gave a first comprehensive insight into the visual processing of cats. But while mice have a low visual acuity (Prusky & Douglas 2004) they became more and more popular and are nowadays an often-used model organism in visual research. The advantages overcome the disadvantage of low visual acuity. One advantage is the availability of transgenic mice, to perform various manipulations on the genome, like creating knockout-mice or the labeling of specific cell types. In addition, their housing is comparably easy and cheap. Furthermore, their short maturation and reproduction cycle allows faster and stable breeding (Huberman & Niell 2011). Besides that, there is a wide range of established behavioral tasks for mice, which rely on the visual system, also presented in this thesis.

1.4. Optokinetic nystagmus

Visually evoked eye movement is an important phenomenon in mammalian vision (Alpern 1972). The movement is a mechanism that helps to stabilize the perceived image by movements in the horizontal axis (Leigh and Zee 2006). To induce this reflex, it is suggested to use a repetitive pattern, as seen in different stimuli. The optokinetic following is reported for stimuli consisting out of stripes (Thaung et al. 2012), sine-wave Gabor patches (Prusky et al. 2014) and random-dot stimuli (Dubois and Collewijn 1979).

The optokinetic nystagmus can be measured directly by eye-tracking. Some species show an optomotor response to a drifting stimulus. This is defined by following movements, performed by the head instead of the pupil. This behavior is shown among others in invertebrates (Collett 1980), zebrafishes (Orger & Baier 2005) or mice (Prusky et al. 2014). A study to compare the optokinetic nystagmus with the optomotor response in mice showed that both responses are linearly comparable (Kretschmer et al. 2015). In this study, the same set of stimuli was used to record the optokinetic reflex and the optomotor response (Figure 1.4). In this experiment, the optomotor response was measured in an optomotor response box (Kretschmer 2013). In addition, a variation of this setup with an assembly to head-restrained mice and the addition of an eye-tracking camera was used. It was shown, that the movement of the pupil and the head movement are both positively correlated with the perceivability of a stimulus. The optokinetic nystagmus, together with an approach in operant conditioning allows psychophysical investigations in mice.

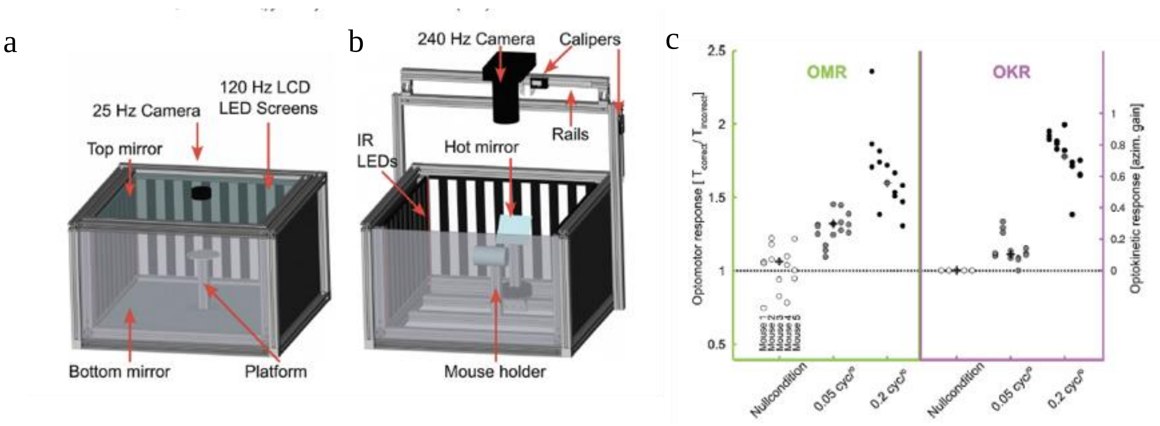


Figure 1.4: Correlation between optomotor response and optokinetic response in mice (adapted from Kretschmer 2015)

a) Optomotor response box, containing four screens, arranged in a square with a platform in the middle on which the mouse is placed.

b) Variant of the optomotor box for head-restrained mice to measure the optokinetic reflex. The camera records the eye over a mirror, which is placed in front of the mouse holder. The stimulus presentation is the same as in the optomotor box.

c) Results of recordings in both boxes. The optomotor response shows an increase from a spatial frequency of 0.05 cycles per degree to 0.2 cycles per degree (green). The same increase can be seen for the optokinetic response (purple)

1.5. Operant conditioning

Burrhus Frederic Skinner was an US-American psychologist, who coined the concept of operant conditioning. With the invention of the Skinner-box, the history of goal-oriented animal training started (Figure 1.5). The general idea of operant conditioning follows the basic concepts of reinforcement and punishment.

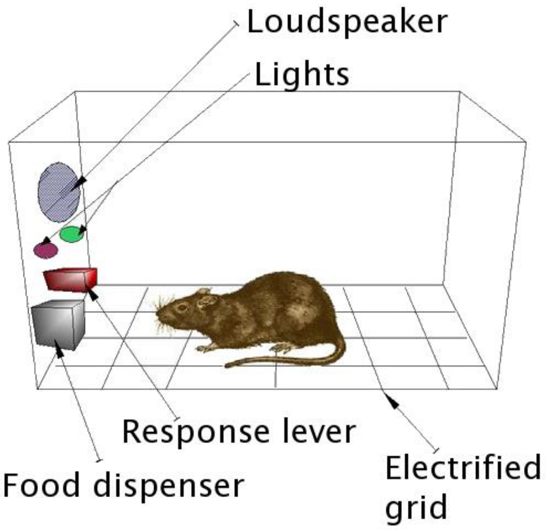


Figure 1.5: Example of a Skinner Box. *The box contains a food dispenser, which delivers the reward for the animal, a response lever, which needs to be touched by the animal and different stimulus presentation parts. In this case, the stimulus is presented via a green and red light, as well as via a loudspeaker. Every correct response leads to a reward, while every wrong response leads to punishment in the form of a foot shock (© Wikimedia)*

The animal gets rewarded if it shows a specific behavior. In terms of operant conditioning, the animal is able to control its behavior, compared to classical conditioning. The pawlowian dog, as a famous example for classical conditioning, showed a reflex behavior as a response to a stimulus. The dog was trained to link the application of food (unconditioned stimulus) with the ring of a bell (conditioned stimulus). The following salivation, which was induced by the unconditioned stimulus, was then also induced by the ring of a bell. The advantage of operant conditioning is that the response to the stimulus is not a reflex, but controlled behavior by the trained animal.

One example for controlled behavior, is pressing a lever, when the green light turns on. This is called positive reinforcement. In the case of unreferred behavior, for example, pressing the lever, when the red light turns on, a slight electric foot shock is applied. This is an example of positive punishment. In this context, the term “positive” can be misleading. It has to be mentioned, that the term “positive” does not refer to the animal’s rating of the situation, but is a descriptive term for the applied consequence. Negative reinforcements mean that punishment does not happen. This would mean, that an aversive stimulus is consequently present until the animal shows the desired behavior. Negative punishment would mean in this context, that a positive stimulus stays absent. In the context of the skinner box, this would be a non-rewarded trial, if the animal does not show the desired behavior (see Table 1).

Table 1: Overview of the different kinds of reinforcement and punishment. *The table shows an overview of examples for positive and negative reinforcement as well as positive and negative punishment*

	Reinforcement	Punishment
Positive	An appetitive stimulus is applied, e.g. a reward	An aversive stimulus is applied, e.g. a foot shock
Negative	An aversive stimulus is removed, e.g. a turned on light turns off	An appetitive stimulus is removed, e.g. a reward is not delivered

Depending on the experimental design, it is possible to do a continuous or partial reinforcement. In a continuous reinforcement paradigm, the reinforcement follows every time when the desired or undesired behavior is shown. In a partial reinforcement setting, the behavior is only reinforced in a fraction of the trials. The decision between these two approaches relies on the general setting of the behavioral experiment. For example, if animals have to be rewarded with sweet milk (Bussey et al. 2008) it would be reasonable to reduce the number of rewards to avoid health problems. Approaches in operant conditioning are well-suited to investigate the functionality of the visual system. For our purpose, we want to train mice to show a specific response after perceiving a stimulus.

This gives us needful information about the capability of the mouse' visual system from a functional point of view.

1.6. Psychophysics

The historical definition of psychophysics says, that psychophysics is "the theory of the relation between body and soul" (Fechner 1858). Therefore, the goal of psychophysics is to show the relation between the perception of the stimulus and its physical parameters. Psychophysics is still a recent field of research, which has a focus on the link between physiological (internal psychophysics) and psychological (external psychophysics) effects. Thus, the interaction between a stimulus and the neuronal system is the central element of psychophysical research.

One prominent example of a psychophysical experiment is the two-point discrimination experiment (Weber 1835). In this sensory experiment, two different points of the upper skin layer are touched. If the two points are close to each other, it is possible that the participant perceives the two touches just as one touch. By increasing the distance between the two touched points, the probability increases that the two touches are perceived and the participant can discriminate between the two touched points. This rather simple experimental approach illustrated the idea behind the field of psychophysics. The intensity of a stimulus, in this example the distance between the two touch points on the skin, has a direct link to the perception of a stimulus and shows the existence of tactile receptive fields.

An early postulation of a psychophysical law was made by Heinrich Weber. He postulated that the ratio k between the change in the stimulus intensity ΔR and the absolute stimulus intensity R is constant in terms of discrimination (Eq. 1):

$$\frac{\Delta R}{R} = k = constant \qquad (1)$$

To visualize this equation, one can imagine carrying different weights. If there is a tiny amount of weight, for example, 2g, it will be easy to distinguish it from a weight with 3g. However, if one would compare a weight of 1.002g to a weight of 1.003g it would be hardly possible to detect a difference.

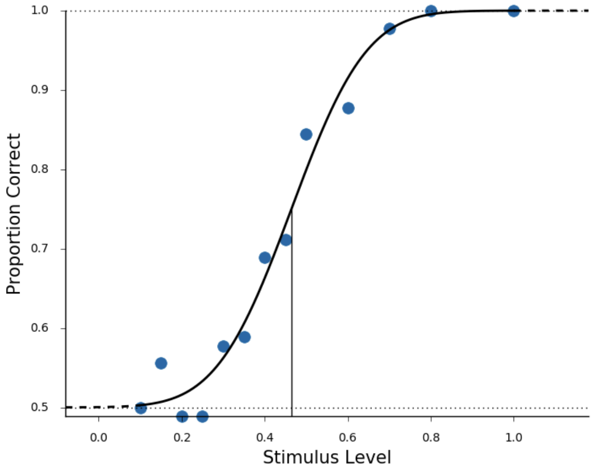


Figure 1.6: Psychometric curve created with Psignifit. *The psychometric curve shows the proportion correct over a generic stimulus level. Single recordings are shown (blue dots) as well as a sigmoid fit (black solid line). The lower dashed line indicates the guess rate at 0.5, while the upper dashed line indicates the saturation at a proportion correct of 1.*

Staying at the example of two different weights, which has to be discriminated, a psychometric curve can be recorded, if the weight varies over different values. If the participant has to decide, which weight is heavier, while there is no or an undetectable difference, the participant will perform on the guess level. It can be expected that the proportion correct is 0.5 if the participant can just guess, which one of two weights is heavier, when there is no difference. This does not apply when the participant has to decide between more than two weights. The guess rate is changing by 1/n. This has also an influence on the discrimination threshold, which is defined to be 50 % above the guess rate (Figure 1.6).

In terms of a task with two options, this is a proportion correct 0.75. The goal of a psychophysical experiment is to measure a psychometric curve. In a detection task, where a participant decides if a stimulus is present or not, the same psychometric curve applies, but with different key values. A Go/NoGo-task, which is needful when the detectability is measured, provides other analysis options than a two-alternative forced-choice task. In a Go/NoGo task, it is only measured if there was a response or not, which is well-suited for detection task. In this case, it is possible to include a receiver-operator-characteristic curve, which compares the number of correct responses and false alarms, which are responses when the stimulus is absent. This experimental design does not fit, if a participant is asked to respond to differences between stimuli. There is no guess rate, since it is likely that a participant is not responding if no stimulus is present. The detection threshold is reached at the stimulus level where the participant classifies the stimulus in 50 % of trials as present.

Another approach is a dynamic stimulus intensity or difference included in the experimental design. The stimulus intensity is, in a so-called staircase procedure, depending on the performance of a participant. For a detection task, this would mean, that the intensity is increasing until the participant responses. After the response indicated that there is a stimulus present, the intensity is decreased until the participant does not respond and indicates that there is no present stimulus. The output of this approach is not a homogenous psychometric curve, in terms of an independent distributed number of repeats over all stimulus levels. The staircase procedure gives, according to the ratio of the step size between the increase and decrease of the stimulus intensity, the desired key values. This would lead to a psychometric curve with a large number of repeats close to the desired threshold, but less repeats at the guess rate or the saturation.

1.7. Animal welfare

Sixty years ago, William M.S. Russel and Rex Burch laid the foundation for today's code of animal welfare. In their book The Principles of Humane Experimental Technique’, they concluded their findings to define the 3R-principles to remove “inhumanity from animal experiments” (Russel et al. 1959).

The first R stands for Replacement. Thus, there is a question if a living and conscious animal can be replaced by other materials. It can be divided into complete and partial replacements. A complete replacement would lead to replacing animals with human volunteers, the use of models on a computational or mathematical basis or the transfer of experiments to cell cultures and tissues. A partial replacement would mean to transfer animal experiments from higher animals, like mammals to invertebrates, e.g. Drosophila melanogaster, due to their deviant experience of suffering, mentioned in ‘The Animals (scientific procedures) Act 1986 (Hollands 1986)’.

The second R for Reduction indicates a careful design of an animal experiment to reduce the needed number of animals. On the one hand, this principle asks for the reduction to the minimum of used animals, on the other hand, the reproducibility has to be given. Another point, which can reduce the number of used animals is the amount of data collected from one animal. The more data one animal can produce, the fewer animals are needed for statistical relevance. A third point to reduce the number of animals and/or experiments is to share data with other researchers. Here it has to be pointed out, that all experiments have to be reproducible.

The third principle of the 3R-principle is Refinement. To refine an experiment every step has to be investigated. Typical questions are, if the suffering of the animal can be decreased or if pain can be avoided. Furthermore, refinement means to reinforce the natural behavior of an animal from the housing to the experimental treatment. The willingness of an animal to cooperate in the experiment is often linked to a treatment, which should affect the animal in the least harmful way.

The application of the 3R principles provides an important guideline for experimental work with animals. The relevance of animal welfare is one of the driving forces in this thesis. We want to propose a method, which leads to a high quality in standardization, which leads to a reduction of needed animals as well as a minimization of suffering for the animals, which fulfills the demand for refinement and reproducibility.

1.8. Goal

All experiments described in this thesis were conducted in the context of basic research on the visual system. My main goal was to contribute to basic research in order to promote medical research in related fields and to test differences between artificial and nature-inspired stimuli with respect to their perception and how it depends on the statistics of the image parameters like orientation of edges and their spatial frequencies To establish an accessible and reproducible framework of different tools, the technical methods that are presented in this thesis were developed with the focus on usability and flexibility. After the establishment of the methods, they can also be used for different mouse models, which are highly relevant in medical research. The second chapter will focus on a touchscreen chamber, which is python-based and provides a highly effective and automated, but also highly standardized operant conditioning.

Another method, from the view of the functional level, is the measurement of the optomotor response in mice, which is a method that does not need proper animal training and runs “out-of-the-box”. These two methods, either a touchscreen chamber or an optomotor response box, are already useful to investigate the function of animal behavior on the level of perception and cognition. But there is also a transition from the functional level of behavior to the physiological side. This thesis also tries to provide a more holistic view by shining light on the missing links. Next to the use of the well-known and often used Gabor patch gratings, the before mentioned experiments were also conducted using nature-inspired stimuli, so-called Motion Clouds.

As the last point, the experiments in this thesis have the goal to minimize the suffering of animals in animal experiments. In light of the complexity to study a living organism, it should be always the goal to optimize experiments to reduce the number of animals and their individual stress levels. Besides that, we also propose a concept for a practical course to educate future neuroscientists with emphasizing behavioral experiments as a central point of neuroscientific research.

The goals of the thesis can be described as followed:

1) Provide a flexible and comprehensive touchscreen chamber for operant conditioning

Compared to a Skinner Box, a touchscreen chamber provides the flexibility to use a various range of different experimental paradigms. Operant conditioning was used in the investigations of the visual system in various studies, with different experimental approaches, which relied on different interplays of reinforcement and punishment. In this approach, we want to test freely-behaving mice without a strong aversive stimulus like the exposition to water in a water maze task. In addition, we would like to provide a translational open-source framework, which also takes the FAIR-principles for data management into account.

2) Test the functional effect of the overrepresentation of neurons tuned to cardinal orientations

To test if there is a behavioral effect of the overrepresentation of cardinal tuned neurons, as well as the overrepresentation of cardinal orientated edges, we designed a parallel visual discrimination task. Furthermore, we tested, the capability of discriminating orientations in a visual discrimination task, following the idea of a staircase procedure. This also leads to the idea, if there is an effect, to test if it is possible to decrease the size of this effect due to a specific training.

3) Test the perception of natural-inspired stimuli in the touchscreen chamber

To gain an insight into the perception of natural-inspired stimuli we transferred the established paradigms to another set of stimuli. Therefore, we used stimuli, which provide different bandwidths in terms of orientations and spatial frequencies. With the approach we used, we are able to test a hypothesis from neuronal models in functional behavior. For this goal we used an orientation discrimination and a spatial frequency discrimination task.

4) Utilize the touchscreen chamber to teach neuroscience

To apply our methods in a larger context, we propose the concept for a practical course in the field of neuroscience. In this course, participants learn how to conduct an operant conditioning experiment, but also how to be aware of the keypoints of animal welfare and health monitoring.

Chapter 2: A python-based touchscreen chamber for operant conditioning

2.1. Abstract

The demand for behavioral experiments in neuroscience to test neuronal findings on the functional level raises the need for modern and affordable methods. In the field of operant conditioning, following the basic principle of a Skinner Box, touchscreen chambers are a helpful tool. They provide an automatized and standardized animal training to produce valid data, which is also in line with the idea of animal welfare. The disadvantage of touchscreen chambers is, that they are either quite expensive or have a rather low functionality.

We propose here a python-based touchscreen chamber, which provides a high flexibility, a high usability and comprehensive functionality. Therefore, the psychophysics-toolbox PsychoPy is used, which is even accessible without profound programming knowledge. As an open-source approach it is freely available for the whole scientific community to conduct experiments in the visual system or in terms of multimodal approaches. This could lead to a common standard of produced data, which follows the FAIR-principles for data management. The touchscreen chamber is used for the pretraining, in which animals have to learn the basic principle of touching a stimulus in order to get a reward as well as for an experimental orientation discrimination task. The presented touchscreen chamber is compared to other approaches with a view on their usability and experimental outcome. The 2-alternatives force choice orientation discrimination task is acquired in 8 to 15 sessions. In addition, a staircase procedure is implemented and tested. In this procedure, it is possible to get an estimate of psychophysical thresholds within one session per animal by a dynamic orientation difference according to the performance of the animal.

2.2. Introduction

2.2.1. Motivation

Animal training is one of the most sensitive parts of an animal experiment. On the functional level, it is possible to investigate a large variety of effects linked to other neuroscientific approaches, as behavioral phenotype screenings for newly created mouse lines or for specific knock-out models. Nevertheless, it is also a helpful tool to investigate principal brain functions and support hypotheses from other neuroscientific fields. To achieve a level of certainty and usability, behavioral experiments should follow the following rules (Mar et al. 2013):

1) Standardized and automatized experiments
2) Non-aversive and low stress
3) Translational
4) Asses multiple cognitive domains
5) Flexibility to use different paradigms
6) Quantitative measurements
7) Low costs

These rules enable a high level of certainty and usability, but another important point is to take the principles of animal welfare into account. To this end, the 3R-rules were established (Russel et al. 1959, Törnqvist et al. 2014, Jing 2017, Manciocco et al. 2009). These rules imply for every animal experiment to consider a reduction of animal numbers to the minimum, the refinement of methods to support the animals' wellbeing, and a replacement of the use of animals in experiments, whenever possible. Additionally, there is an ongoing discussion about introducing a fourth "R" for responsibility; this last aspect targets the ethical responsibility owned by the experimenter (Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V., 2016).

The presented approach also gives the opportunity to create data according to the FAIR-principles as a common standard. FAIR is an acronym for findability, accessibility, interoperability and reusability.

In the following chapter, the current state-of-the-art is described and a detailed view on how to fulfill the different requirements is given. The requirements are described in more detail, but also how they fit the animal welfare requirements.

The following chapters will also introduce different possible experimental paradigms that are realized within a framework of python scripts, which can be used universally and as far as possible also independently from specific hardware requirements.

2.2.2. Standardized and automated experiments

To compare single trials with each other and see effects over several animals it is highly recommended to have a very low variability over several experiments. Therefore, the parameters have to be chosen carefully. In terms of operant conditioning, this means to optimize experimental parameters like the size of the stimuli, the intertrial-intervals (ITI), the number of trials or the reduction of cognitive bias (Bussey et al. 2008).

A high standardization opens the door to a reliable automatization. Some experimental paradigms, for example, the virtual water maze task (Prusky et al. 2000, Brandeis et al. 1989, Wang et al. 2016) needs an experimenter that places the mouse or rat back to the starting position after every single trial. The stimulus size, intertrial interval, the number of trials and a spontaneous stimulus-dependent bias were tested by Bussey et al. (2008). The stimulus sizes tested in rats were 2.5 cm^2, 3.5 cm^2, 4.5 cm^2 and 5.5 cm^2 (equivalent to a square-shaped stimulus with a length of 1.59 cm, 1.87 cm, 2.12 cm, and 2.35 cm). It was shown that a larger stimulus increases the slope of the learning curve, which leads to the conclusion that the training is influenced positively by a large stimulus (Figure 2.1).

Another question that was addressed, is the experimental design in terms of the initiation of a trial, a forced intertrial-interval and the number of trials. In principle, it was shown that the initiation of a trial by the animal is preferable over non-initiated trials and that a long ITI also increases the slope of the learning curve. The number of trials also plays a role. The authors showed that a higher number of trials is beneficial for learning. To test for interactions between these variables, different combinations of experimental settings were tested. As suggested by the previous results, a combination of 60 trials with a long

ITI, which were initiated by the animal, showed the best results within the tested combinations (Figure 2.1)

This previously tested parameters give already critical hints for a successful experimental design.

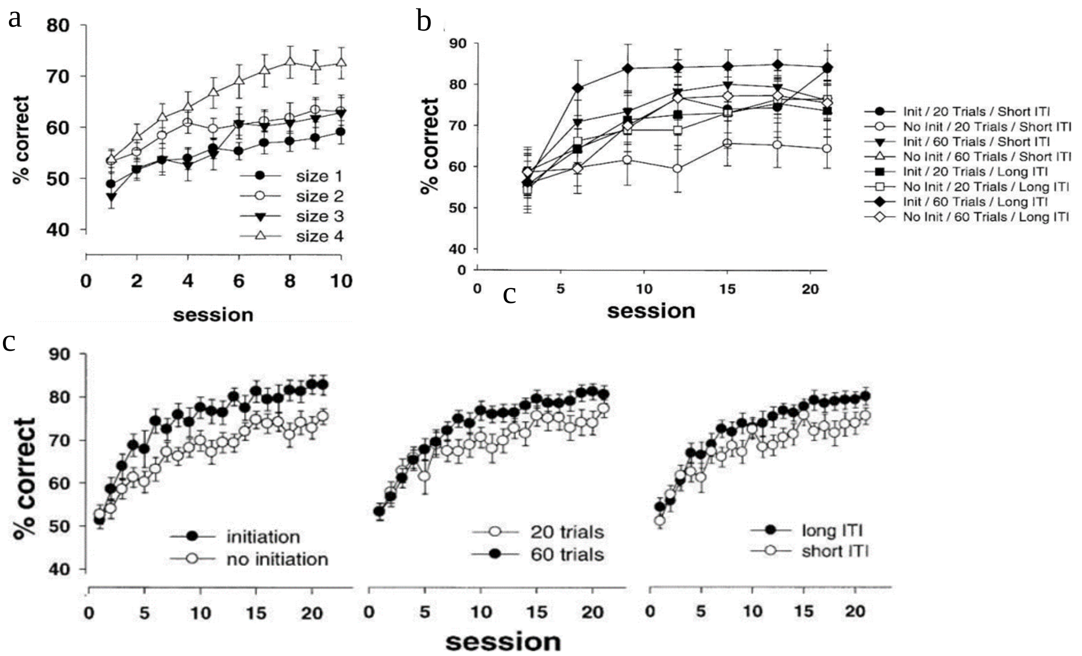


Figure 2.1: Performance Curves for different settings in the Bussey-Saksida Touchscreen Chamber

a) Percentage of correct trials over sessions at different stimulus sizes

b) Percentage of correct trials over sessions at different settings for the trial initiation, trials per session and length of the intertrial interval

c) Results of more specific testing of the parameters trial initiation, trials per session and length of the intertrial interval

2.2.3. State of the art

Automatic operant conditioning is already established in different contexts. Leising et al. (2013) presented an automatized and touchscreen-based operant behavior chamber which used an iPad for stimulus presentation and touch responses for rats (Figure 2.2). This test chamber had a rectangular shape and measured 30.5 cm x 24.1 cm x 29.2 cm (length x width x height) and was made out of clear plexiglass connected by aluminum panels. As a reward, a liquid sucrose solution (16 %) was given. The iPad was used as an additional monitor of a computer by using extra software: Remote Desktop Protocol (Mochasoft Apps, Blokhus, Denmark) from the Appstore of Apple (Cupertino, USA). All other software was custom-written in Microsoft Visual Basic 6.0. The utilized iPad used a capacitive touchscreen. Capacitive touchscreens react to touches instead of pressure by a change in the surface capacity.

In this setup, two out of four rats failed to learn a visual discrimination task with a fixed length of stimulus presentation, when they did not get differential feedbacks on their response. For those rats which learned the task, the performance increased after 12 days to a maximum of 70 % to 85 %, respectively. In another experiment, the same setup was used but with correction trials. So, if the rat replied incorrectly, it got another chance to reply to the identical stimulus as before. After the refinement of the procedure, all rats were able to learn the task.

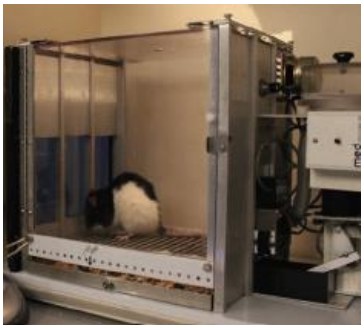
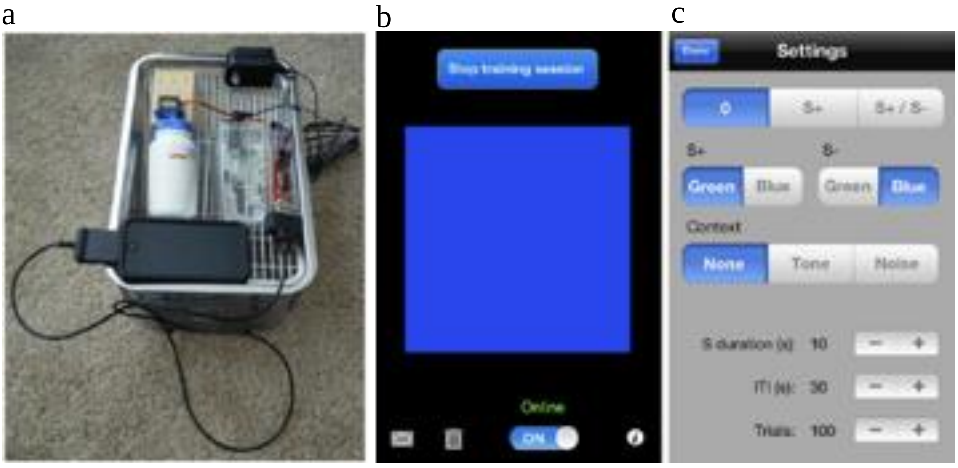


Figure 2.2: Touchscreen Chamber by Leising et al. 2013. *The rectangular area for rats is shown. On the right side, the reward delivery system is visible. The screen on the left side is an attached Apple iPad*

Another approach utilized an iPod Touch in connection to an Arduino Uno microcontroller (Pineño 2013). All the components were attached to a home cage of a rodent (Figure 2.3). In this case, the stimulus presentation was computed by the iPod Touch. In comparison to the approach shown in the first part of this chapter, it did not need an additional computer. Therefore, the author developed an app, running on apple devices to present a simple stimulus, e.g. different colored rectangles. The iPod Touch sent then just a single value to the Arduino to trigger a servo motor, which released the water reward. By the end of the experiment, the saved data were exported via email to another computer for further analysis. By the publication date, the app was able to conduct visual discrimination tasks with their color as the changing variable. The tested rats in this paradigm showed up to 80 responses in 50 minutes. Unfortunately, the authors did not provide performance values or learning curves for this approach, but they pointed out the low costs of the setup which was valued at less than 300 USD.



S duration (s): 10
ITI (s): 30
Trials: 100

Figure 2.3: Touchscreen Chamber approach by Pineño 2013.

a) Overview of the touchscreen apparatus on top of a homecage.

b) Stimulus presentation on the developed app.

c) Graphical User Interface on the developed app

The third approach (O’Leary et al. 2018, Figure 2.4) is a touchscreen chamber using a Raspberry Pi and a fitting touchscreen (also by Raspberry). It was used to train rats on touching a white rectangle. Responses were food-rewarded, while the food delivery system was controlled by a servo motor. A piezo buzzer provides an auditory cue when the food pellet is delivered. The arrangement can be placed flexibly in a home cage. The costs for this operant conditioning box was calculated to be at 158 USD. Different training stages were compared to the results achieved in commercial operant conditioning boxes by Med Associates. In this custom operant conditioning box, animals performed only a third of the number of trials achieved in commercial ones in a very specific task. Overall the animals performed around 60 trials in 60 minutes. This approach is not suited for mice, since the utilized touchscreen uses a capacitive screen. To our knowledge, capacitive screens are not sufficient to detect touches of mice.

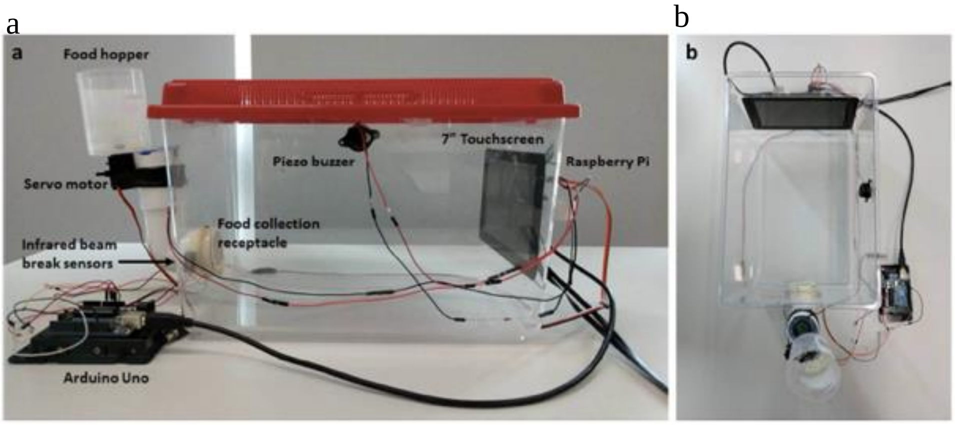


Figure 2.4: Touchscreen Chamber by O´Leary et al. 2018

a) View from the side onto a homecage assembled with a touchscreen and the reward delivery system.

b) View from above of an opened home cage including the touchscreen and the reward delivery system.

The most comprehensive, but also most expensive solution, was the Bussey-Saksida touchscreen chamber (Bussey et al. 1994, Bussey et al. 2008, Bussey et al. 2012, Horner et al. 2013). This version of the touchscreen chamber was not placed in a house cage but had a dedicated trapezoidal area for the mouse training (Figure 2.5). Therefore, the mouse had to be placed individually in the touchscreen chamber. It included a 12.1-inch infrared touchscreen display for mice. There was also a larger version for rats with a 15.0-inch infrared touchscreen display. For mice, the chamber has dimensions of 18 cm x 24 cm (at the screen)/6 cm (at the reward magazine) x 20 cm (length x width x height). The screen was masked with a paradigm-specific plastic plate, which allowed the animal to only touch the stimuli and hides other areas of the screen. Another infrared-beam in front of the reward delivery magazine indicated if the animal was collecting the reward. It runs on the ABET II Software (Campden Instruments Ltd., Loughborough, United Kingdom). In this setup, mice were described to learn to perform in a visual discrimination task within 9 to 18 sessions (Bussey et al. 2001).

Figure 2.5: Bussey-Saksida Touchscreen Chamber (Horner et al. 2013). *An opened touchscreen chamber is shown. In the experimental setting, the open side is closed. The reward delivery system is shown as well as the touchscreen. The area of the touchscreen, which does not show the stimulus is masked.*

All in all, there are already approaches to costumize touchscreen chambers. It is remarkable, that the operant conditioning boxes are dominantly made for rats. Except the Bussey-Saksida Touchscreen Chamber, all chambers are restricted to very limited experimental designs. This missing flexibility is a problem we would like to tackle with our approach. For a general overview of the presented operant conditioning approaches, see Table 2.

Table 2: Overview of different Touchscreen Chambers. *The central characteristics of the presented touchscreen chamber approaches are shown in the table below, including the sessions animals needed to reach the criterion, the technique used in the display to register a touch, the species used, the geometrical shape of die chamber and the costs*

Reference	Sessions to the criterion in VD-task	Display	Reported Species	Shape	Costs
O’leary et al. 2018	-	Capacitive	Rats	Rectangular	158 USD
Leising et al. 2013	12	Capacitive	Rats	Rectangular	-
Pineño 2013	-	Capacitive	Rats	Rectangular	300 USD
Bussey et al. 2008	9 to 18	Infrared	Rats Mice	Trapezoidal	14.000 USD

2.2.4. Non-aversive and low stress

The influence of stress in animal experiments is a largely discussed issue. Several points have to be taken into consideration. First, stress influences the results of an experiment (Korte & De Boer. 2003, Graybeal et al. 2014). It has been shown that acute stress decreases the explorative behavior of rats in an elevated plus-maze. Animals without any external stressors spent around 40 % of the time of the experiment in an open arm of the maze. A group of animals that were stressed with a foot shock before the experiment showed a significant decrease to less than 10 % of their time on the first day of this treatment. The animals adapted to stress, so they recovered the time spent in an open arm within seven days of treatment to a non-significant decrease of time spent in an open arm (Korte & De Boer 2003). Graybeal et al. 2014 compared different mouse strains in regard to their ability to learn under a stress treatment regime, where the mice had to do a forced swim test before they were transferred to a touchscreen chamber. DBA/2J mice were not affected by the stress.

The commonly used C57BL/6J strain was affected in a paradigm-specific way, like worse performance in the reversal learning paradigm under stress. Hölscher et al. 1999 considered animals, that are used to handling before the experiment, as less stress-harmed. In their study, they compared animals, that were habituated to handling, to those who were not. In their spatial working memory-paradigm, the animals performed worse, when they were not habituated to handling, which the authors considered to be a sign of increased stress. Another approach to measuring stress in animals is to analyze the heart rate (Stiedl et al. 1999). In this study, C57BL/6J mice showed a stronger increase in heart rate than DBA/2J mice, when they were fear-conditioned. This was also correlated to an increase in time freezing and a decrease in explorative behavior.

Beside experimentally induced stress, animal housing, and handling also seem to be stressors for mice (Tuli et al. 1995). Therefore, the corticosterone level was measured in different experimental groups. After the transport from an animal facility to another room, the animals had an increased level of corticosterone. The authors concluded that there are many stressors, which can increase corticosterone like a change in the environment or new cages. Corticosterone can also be collected from fecal samples (Malien et al. 2016). They tested specifically for a change in corticosterone in touchscreen-based setups. They showed that a higher corticosterone level is not correlated to the learning performance or even increased the responses to the stimulus. Thus, it can be concluded that elevated corticosterone is not a specific marker for distress, but can also be positive eustress, which was also found in other species when they show increased mating behavior (Ruploh et al. 2015).

Another study, also investigating the learning ability of rodents in touchscreen chambers, did not indicate a change in the learning rate (Martis et al. 2018). In summary, the effect of stress depends on the rodent strain and the specific experimental paradigm. As a general concept, it can be concluded that handling stress should be minimized, while positive stress, like environmental enrichment, can be a positive factor for animal training, also in terms of reduction and refinement (see **Animal welfare**).

2.2.5. Translationality

Translational research has an outstanding relevance for the understanding of different diseases (Drolet & Lorenzi 2011). Using comparable paradigms helps on the one hand to identify the characteristics of symptoms in mouse models, on the other hand, it can lead to the identification of similar symptoms in clinical research. Behavioral experiments in the visual system are in most cases a non-invasive procedure for human participants. A method with high throughput can help to characterize more mouse strains to obtain a deeper knowledge of the clinical relevance. The behavioral phenotyping is already an important topic in neuroscience. Touchscreen based experiments were performed in different mouse strains linked with neurodegenerative diseases and molecular malfunctions in the brain. For example, a study in a mouse line, with an impaired metabotropic glutamate receptor 5 showed a decreased capability for reversal learning in a touchscreen chamber (Lim et al. 2019). The use of a touchscreen chamber is critical in this approach, since the metabotropic glutamate receptor 5 is also involved in stress responses. In a Skinner box, which is also applying foot shocks, the increase in stress can distort behavioral findings in this specific setting. Another experiment in a mouse strain, associated with schizophrenia, revealed an impaired performance in an attention task, which was performed in a touchscreen chamber as well (Nilsson et al. 2016). The touchscreen chamber is not limited to different mouse models, but can also be used for pharmacological studies to test the effect of a certain drug. A study by Heath et al. 2015 revealed the effect of different amphetamine doses on the motivation of mice in a touchscreen-based task. The touchscreen chamber is a viable method for a wide range of demands. It can cover several parts from basic research, over behavioral phenotyping of mice with different genetic backgrounds, to the application of different pharmakon. This fulfills the requirement of a translational method and leads to questions about the reproducibility of experiments.

2.2.6. Reproducibility

Monya Baker asked 1.500 scientists about reproducibility (Baker 2016). With 52 % more than every second scientists agreed, that there is a reproducibility crisis in science. Around 75 % of all biologists, who responded to the survey made the statement that they already failed in reproducing a colleagues experiments. Remarkably, 90 % of the asked scientists responded that one of the main issues in the reproduction of experiments is the lack in an accurate reporting of conducted experiments. Specifically, the problems in a behavioral research are shown by a cross-laboratory study by Mandillo et al. 2008., framed by a European project called EUMORPHIA. They tried to establish similar standard operating procedures to conduct the experiments at four different places. By this approach, they managed to have qualitatively similar results in 66 % to 100 % of the laboratories in several behavioral paradigms.

The challenge of reproducible experiments is also targeted in this thesis. To establish a method, which provides the possibility of a high reproducibility is to reduce the influence of the experimenter. Therefore, we try to reduce handling times and the contact to the animal by an automatized approach. Going back to Baker 2016, one problem for the reproducibility of experiments is the lack of experimental information. We want to take the FAIR-principles into account for the data output generated in the presented approach. Therefore, the data needs to be findable, which means that it must be possible to search within the data. They need to be accessible, which means, that they must be archived in an organized way. To avoid storage problems, we aim for data, which is saved in a non-consuming way in terms of memory space. This leads to the third point, to generate interoperable data. Data should be readable by machines, as well as by humans. The reusability of data is management by the storage of metadata.

2.2.7. Assessment multiple cognitive domains

Linked to the requirement to be translational, it is also important to have a method, which is flexible enough to assess multiple cognitive domains, for example perception, attention or memory. Compared to other behavioral experimental approaches like fear conditioning or elevated plus mazes, it is likely that the number of cognitive domains is very restricted. Besides that, depending on the experimental hypothesis it can be hard to bring the results of two different experiments together. So the requirement is to have an approach that gives the possibility to do many different paradigms without using a fundamentally different approach.

2.2.8. Flexibility

Together with the translational character of the method and the ability to assess several cognitive domains in an animal, it already satisfies a certain demand for flexibility. Beyond this, previously presented touchscreen chambers were mainly established for rats. The limiting factor here is the display and the ability to recognize touches on the display, respectively. It has been shown that resistive touchscreen displays are only usable for larger animals like pigeons or rats since they react to pressure (Gibson et al. 2004, Cook et al. 2004). Recent rodent-based operant conditioning systems mainly use capacitive displays. They are more sensitive to touch without any pressure (Reis et al. 2010), but their sensitivity is not sufficient for mice.

The possibility to conduct experiments without the limitations of a restrictive software framework, was an outstanding point for us. Therefore, we use a standalone Python-environment called PsychoPy, which is freely available and open source. By providing the needed functions, it is possible to realize every experimental paradigm, which was used before in touchscreen chambers. Besides the paradigm it also important to have the possibility to use different stimuli in contrast to a predefined set as well as movies. For multimodal approaches, it is also possible to include different auditory cues.

Another requirement was to develop a modular touchscreen chamber, with a fundament of software and hardware solutions, that can be transferred to rats or other animals with a low amount of manipulation or additional costs. A further requirement in flexibility is the transportability of the touchscreen chamber. In terms of different experimental paradigms or cooperations with other laboratories, it can be very useful if the setup can be transported easily.

2.2.9. Quantitative measurements

A behavioral experiment contains a wide range of different readouts, which can reveal differences between controls and experimental groups. In the specific environment of an operant conditioning chamber, there are certain timings which are worth to be recorded. On the one hand, this is the reaction time, which is the time the animal needs to respond to a stimulus after its onset. After the response to a stimulus, there is also the lick delay. This is the time an animal needs to collect the reward or respectively initiate the next trial after a wrong response. These two timings are markers for the attention and motivation of the animal (Humby et al. 2005). Another parameter worth recording is if the animal is responding to the right or left side (Yu et al. 2018). This way, it is possible to identify strongly biased animals or single biased sessions. From this, it can also be derived if the animal is using a strategy by responding alternatingly to the right and left side of the screen. Nevertheless, it is mandatory to save the amount of correct and wrong trials as specific stimulus information. A more sophisticated recording is the tracking of the mouse position and movement speed (Giancardo et al. 2013).

Besides that, it is also important to have proper data management. It is suggested to follow the FAIR principles (Wilkinson et al. 2016). FAIR is an acronym for findable, accessible, interoperable and reusable. These principles contain that the data is uniquely assigned and described. To achieve high accessibility, the data should be human- and machine-readable. The interoperability is reached by creating a dataset that does not require a specific programming language or analysis protocol. To make data reusable it is important to provide enough information within the metadata to be able to reproduce an experiment later on and identify the experimental settings specifically.

2.2.10. Low costs

As shown before the costs for an operant conditioning touchscreen chamber vary from 158 USD up to 14.000 USD. Of course, the price is related to the functionality and the practicability of an operant conditioning touchscreen chamber. Cutting down costs is always an important demand, also if a method should be provided in teaching. Therefore, a goal is to provide an open-source framework in terms of hard- and software. Python is a comprehensive programming language for stimulus presentation, hardware control, and analysis. In comparison to Matlab (The MathWorks Inc., Natick, Massachusetts, USA) it provides similar functions for psychophysics. For the use in psychophysical experiments a specific standalone package called PsychoPy is provided (Peirce 2007, Peirce 2009).

2.2.11. Goal

The goal of this chapter is to provide a comprehensive touchscreen chamber using freely available software to gain a maximum level of flexibility. Therefore, the aims are as following:

1) Concept of a modular hardware

The hardware should be compatible and easy to purchase. The effort to assemble it should be quite low. As an advantageous approach, we aim for a trapezoidal shaped animal area with an infrared touchscreen. The reward delivery system will be made for a liquid reward, because liquid rewards can be used for water-restricted and for food-restricted animals. The touchscreen chamber should be easy to disassemble for cleaning purposes.

2) Programming a comprehensive software framework

The software scripts should deliver all relevant functions in terms of stimulus presentation, computation of touches and the control of the reward delivery system. Therefore, a collection of python scripts and functions shall be programmed and tested for a visual discrimination task.

3) Conduct animal training in a visual discrimination task

The last goal is to confirm the functionality of the touchscreen chamber by conducting pretraining procedures and a visual discrimination task. Therefore, the number of responses by the animals in the pretraining is analyzed as well as the learning curves and the number of trials they performed.

2.3. Methods

2.3.1. Animals

The visual discrimination learning task, as well as the pretraining, was performed in four male C57BL/6 mice, the experiments were started when the animals reached the age of eight weeks. The experiments were authorized in the licenses 84-02.04.2016.A357 and 84-02.05.40.17.055. The data for the analysis of the lick delay and strategies were acquired from another batch of six animals, which started the pretraining at an age of eight weeks as well. This was made, to present a larger dataset. They were bred and kept at the animal facility of the institute (RWTH Aachen University). During the experiment, the animals were kept on a reversed Day/Night-cycle of 12h:12h. The animals were water restricted throughout the experiments. They received water *ad libitum* on one day of the week, on which no experiments were performed. Per day they received at least 1.5 ml of water to maintain their wellbeing. They had access to food *ad libitum* all the time. The mice were weighed and observed before the experiments started.

The animals were scored for deviant behavior to check for stress symptoms, injuries or other relevant symptoms. This includes the activity and movement of the animal, their breathing frequency and skin turgor. Minor impairments were scored with one point, major impairments scored two points. If there were no major impairments observed, but the cumulative score of the minor impairments was between one and four, the animals had to be monitored carefully. In case of a major impairment (two points in one of the categories) or a cumulative score of five or higher, the animal needed to be removed from the experiment and had to be euthanized immediately. At no point of the experiment a euthanasia was conducted.

2.3.2. Setup

The setup consisted of polyvinyl chloride plates, which were linked to each other via screws. The trapezoidal shape was described by several authors (Bussey et al. 2007, Mar et al. 2013). The dimensions were 410 mm x 273 mm for the screen holder. Within the screen holder, an 11-inch display with an infrared touchscreen frame was mounted in front of it with a length of 165 mm (e). The infrared frame sent the input information, i.e. touches by the mouse, via the USB port to the computer system. The infrared frame is crucial, because the touches of mice were not detected by a capacitive or resistive touchscreen. On the opposite of the screen holder, the wall had the dimensions of 47 mm x 273 mm (c) and contained the water delivery spout. The distance between the screen and the water spout is 160 mm (d).

The display did not need to be touch-sensitive. The shape of the screen holder is specifically milled to the used display and touchscreen frame. The speakers were placed next to the screen outside of the animals' area (Figure 2.6).

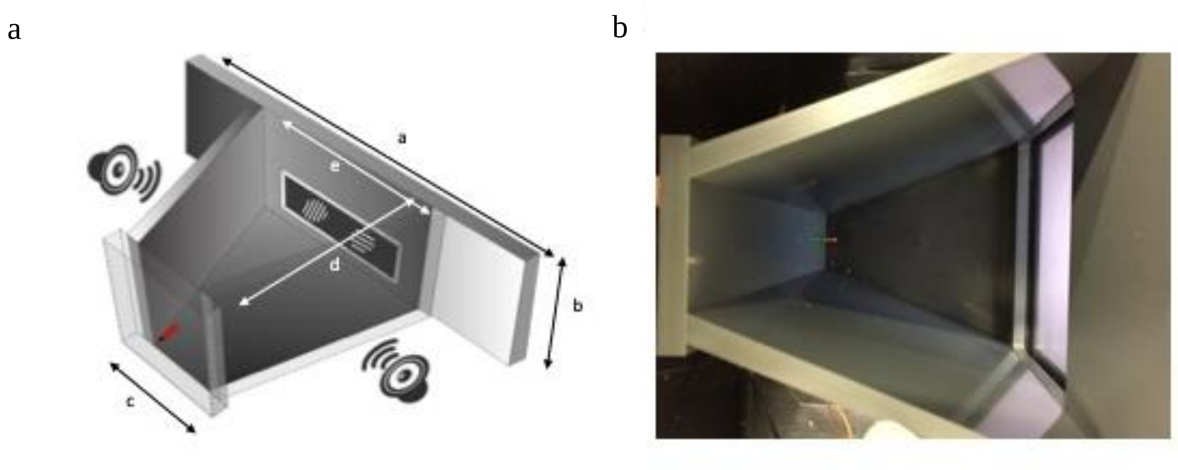


Figure 2.6: Python-based custom-built Touchscreen Chamber

a) Schematic view including the assembled polyvinyl chloride plated, the screen and the loudspeaker

b) Photograph taken from above

The stimuli were presented via a computer with an installation of PsychoPy (v1.83.04, Peirce 2007, Peirce 2009). The display, the touchscreen frame, the data acquisition box (Measurements Computing), the webcam, and the loudspeaker were connected to the computer. Once the screen was touched, the computer received the signal, where the touch happened. The computer triggered an auditory cue to the loudspeaker, dependent on the animal’s response. In case of a correct response, the computer sent a trigger to the data acquisition box to turn on the green LED and played a low-pitched tone on the loudspeakers. If the response was wrong, the computer showed a bright white screen and a distinguishable high-pitched tone. In both cases, the lick detection was activated. When the animal touched the water spout a piezo element was triggered. The signal from the piezo element was usually rather weak, so it needed an amplifier before it was sent to the data acquisition box and the computer readout the input. If the response was correct and the lick detection was triggered, the computer triggered the data acquisition box to send a signal to the relays of the magnetic valve to open it to release a water reward. If the response was incorrect, the animal had to trigger the lick detection, which sent a signal to the computer via the data acquisition box to deactivate the light punishment and initiate the next trial. The webcam recording was started together with the experiment to record the behavior of the mouse during the full experimental session (Figure 2.7).

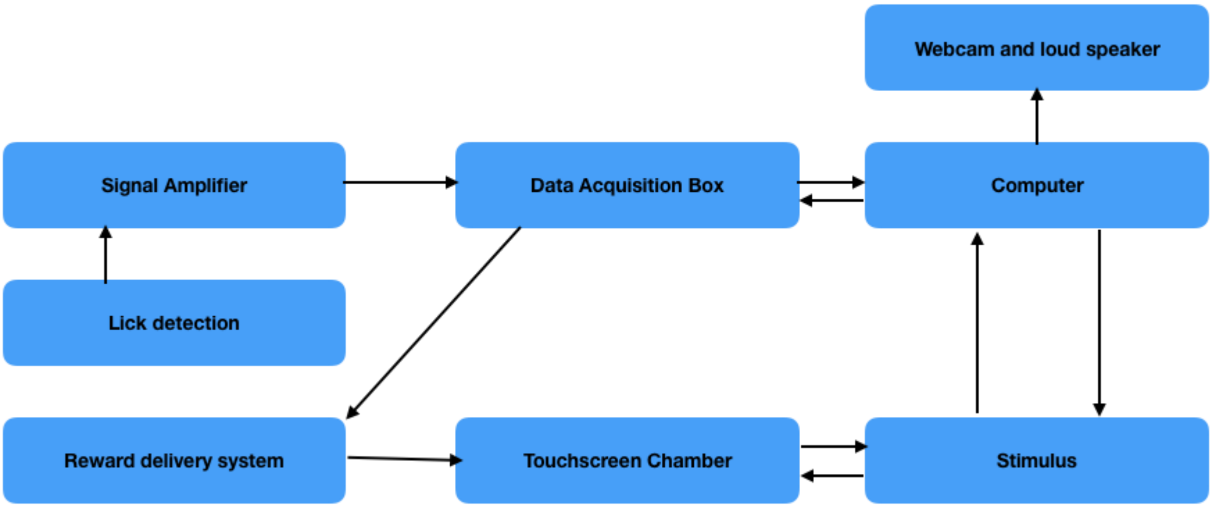


Figure 2.7: Technical workflow within the Touchscreen Chamber *A computer controls all relevant aspects of the touchscreen chamber. Therefore, the computer generates the stimulus via PsychoPy and presents it in the touchscreen chamber. The webcam and the loudspeakers are also controlled by the computer. Once the animal touched the stimulus in the touchscreen chamber, the computer gets the signal and computes if the response was correct or wrong. In both cases, the computer activates the data acquisition box, which is waiting then for a touch at the lick detection. Once the lick detection is touched the amplified signal is transferred to the computer, which activates the reward delivery system and provides a reward to the animal in the touchscreen chamber*

The presented touchscreen chamber costs all in all-around 699 € (Table 3). The most expensive component is a laptop or computer, which is able to execute PsychoPy. In addition, it needs four USB-ports and a video output port, which suits the display. The USB ports are used for the infrared frame, the data acquisition box, the webcam as well as the power supply for the loudspeakers. The data acquisition box by Measurement Computing is mandatory since it works with the universal library used in the stimulus presentation. The costs for the infrared frame include the costs for delivery and customs fee. Thus, these costs can vary due to the country, where the touchscreen chamber is built. All other parts can also be substituted by cheaper parts.

Table 3: Overview of the costs for the python-based touchscreen chamber

Measurement Computing 1208LS (Measurement Computing GmbH)	125 €
Magnet ventile (SMC Pneumatik, 9900001809231)	50 €
PVC plates	30 €
Display 10.1 inch (ELO touch 1002L)	70 €
Infrared Frame (NJY touch, Guangdong, China)	115 €
Clipper Box (Bauhaus)	12 €
Logitech Z120 Speaker (Conrad Electronics)	17 €
Laptop	210 €
Relais	2 €
Cables, screws, tubes, piezo (Conrad electronics)	15 €
Webcam (Logitech C920)	25 €
Dynavox TC-750 Amplifier	28 €
Sum:	699 €

2.3.3. Software

All software scripts are provided in a Git-repository: (https:// https://github.com/BRAINLab-Aachen/Touchscreen-Chamber). The scripts were written with PychoPy 1.83 or Spyder 3.3.6. The used packages were NumPy (Oliphant 2016), Matplotlib (Hunter 2007), Scipy (Bressert 2012), Seaborn (Bisong 2019) and UniversalLibrary.

The basic software framework controlled the stimulus presentation. In the first ten trials, the stimulus presentation was randomized. The rewarded stimulus was either on the right or left side. When the stimulus presentation is activated for two minutes and the infrared touchscreen frame did not receive any responses by the animal, the experiment was finished. When the animal touched the screen, the software compared the position of the touch with the localization of the rewarded stimulus. In case of a correct response, the software sent the trigger to induce the onset of the green light and activates the lick detection with the function “lick_detection()”. This function also triggered a low pitched tone, which was the signal for the animal to collect the reward.

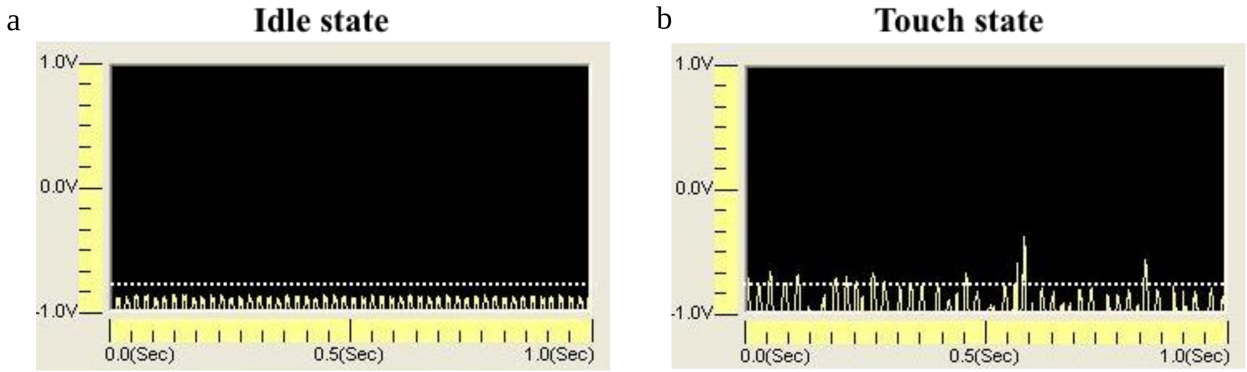


Figure 2.8: Visual readout of the lick detection with InstaCal

a) The amplified noise signal of the piezo element is shown. The yellow trace shows the signal in the idle state. The yellow dashed line indicates the threshold when the piezo element is considered to be touched. b) The amplified signal of the piezo element is shown when it got touched by a mouse over the time course of one second. The threshold (yellow dashed line) is crossed several times, which is considered as a touch by the computer

The lick is detected via the voltage change of the piezo when it gets touched. The threshold is calibrated manually. This can be read out by the UniversalLibrary for Python (Measurement Computing GmbH, Bietigheim-Bissingen, Germany), which works with the data acquisition box. Within the framework, the python script “piezo calibration.py” is provided. It shows the maximum, minimum and means of the voltage trace over 1.000 iterations. In the experimental scripts, the voltage trace is squared and then rooted to exclude negative values. If the voltage trace rises now above the threshold the lick detection is triggered. The function “lick_detection_no_reward()” works the same way, but without the triggering of the magnetic valve and without the green light. In all experimental scripts, it is possible to set a maximum number of trials. The comparison happens after every trial. When the maximum number of trials was reached, the experiment ended. The voltage trace can also be read out by InstaCal (Measurement Computing GmbH, Bietigheim-Bissingen, Germany) to validate the results of the python installation (Figure 2.8).

A schematic view of one full trial is shown in Figure 2.9. The stimulus presentation is normally followed by a response of the animal, which can be either correct or wrong. A correct response is followed by a low pitched tone and the green light to indicate the availability of a reward. The reward is released by triggering the piezo element, which initiates a new trial after the reward is collected. A wrong reward is followed by a light punishment in form of a bright screen. This punishment gets deactivated after the animal touches the water spout and triggers the piezo element. The deactivation of the light punishment initiates the next trial. After a trial, the overall number of trials is checked. If there is a predefined maximum, the sessions stops, when the limit is reached. Another option to end the session is a period of inactivity, which lasts two minute during the stimulus presentation.

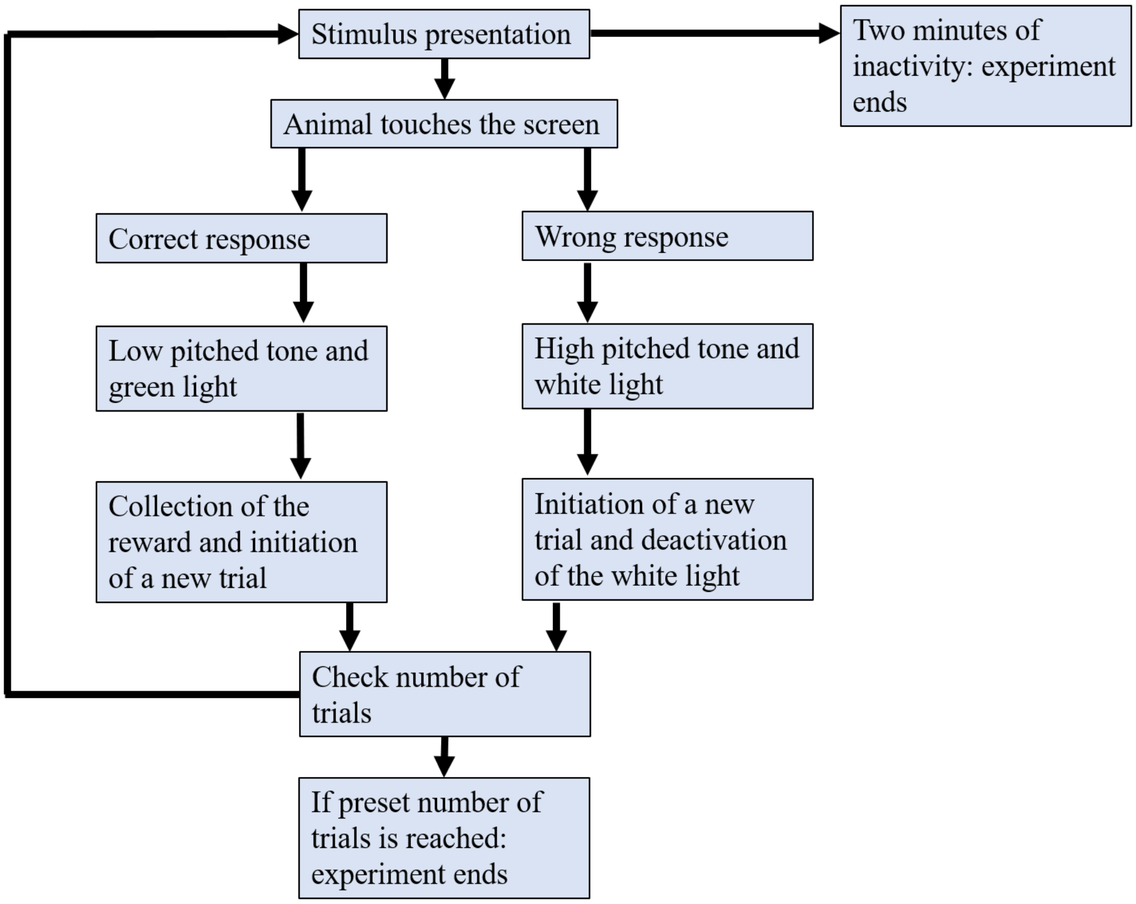


Figure 2.9: Workflow of the stimulus presentation and reaction to correct and wrong trials. *After the screen is touched, it is computed if the response was correct or wrong. A correct response leads to a low pitched tone and a green light above the water spout. A wrong response is followed by a low pitched tone and a white light by the screen. After the animal collected the reward or tries to initiate the next trial, the number of trials is checked. If there is a preset number of trials, it is checked if this amount is reached. If yes, the experiment is finished. Otherwise, the next trial is initiated. If the animal is inactive for a period of two minutes during an active trial, the experiment is finished as well.*

2.3.4. Experimental training

The time schedule of the pretraining depends on the housing of the animals. If there is a dedicated animal facility, it is advisable to move the animals to the place where they are kept during the experiments three days in advance of the experiment. Especially if group housing is needed the animals need a few days to accustom to the group they live in. Then the water restriction protocol can be started 24 hours before the first experiment. In the first phase of pretraining, the animals have to link a green light with a reward. After the training is started, the green light above the water spout turns on. As soon as the mouse collects a drop of water, the light turns off. After a timeout of ten seconds, where the water spout does not provide water when the mouse is licking, the green light turns on again. This is the sign for the mouse, that it can collect another drop of water. This pretraining should last at least for 20 minutes. When the animal collects more than one drop of water per minute on two consecutive sessions, it can proceed to the next stage.

During the second pretraining stage, the mouse has to touch the touchscreen in order to receive a reward. There is a full-screen sine-wave grating shown at this level. For the first session, 45 minutes per mouse are planned. In this time, it is likely that the mouse will touch the screen by chance and collects the reward analog to the habituation phase. If the mouse touches the screen and collects the reward more than once per minute in two consecutive sessions, it can proceed to the experimental training. The time of this phase can be decreased after the first two sessions, according to how fast the mouse is learning. The amount of water, that the animals can collect during this pretraining phases in the box, is not limited.

The experimental training depends on the task the animals will do in the future. In this study, the animals learned to perform in a two-alternative forced-choice task (2AFC). Therefore, the animals were trained with the easiest stimulus set planned for the experiment. Here the animal learned to distinguish between a horizontal and a vertical sinusoidal grating. After the animal reached a performance of 80 % on two consecutive days, the animal can proceed to the staircase procedure for an estimation of behavioral thresholds (Figure 2.10).

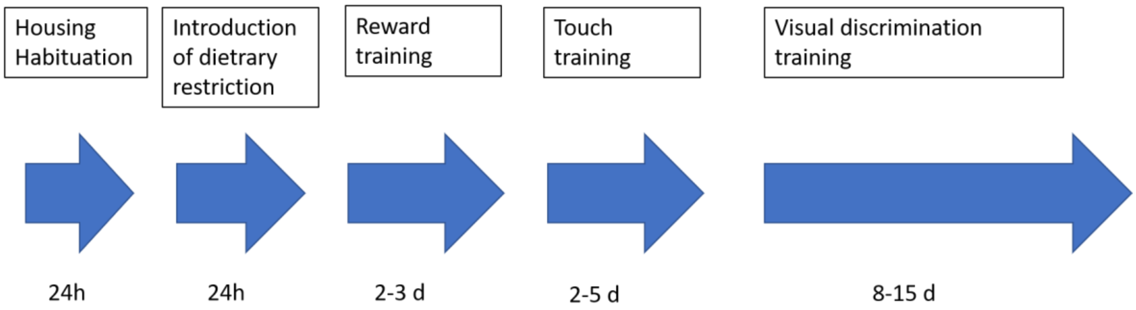


Figure 2.10: Schematic overview of the preatraining and experimental training. *The housing habituation lasted, dependend on the anima facility of an institute for 24h. The introduction of the dietrary restriction, either a food or a water limitation, took 24h as well. After 2 to 3 days the animals understood, that they can collect a reward in the touchscreen chamber. The ability to touch the screen in order to get a reward was archieved after 2 to 5 days. Afterwards the animals were ready for the visual discrimination training, which took 8 to 15 days from a naïve to an expert animal.*

2.3.5. Visual discrimination: Oblique and cardinal

The scripts “vd cardinal.py” and “vd oblique.py” contain an orientation discrimination task, where animals are trained on a cardinally or an obliquely orientated sine wave grating as a rewarded target stimulus. When the animal initiated a trial, there was a short intertrial-interval of 500 ms as a starting delay. This was followed by a visual cue, i.e. a cross. After further 700 ms, the stimuli were shown, so there was the rewarded target stimulus with a horizontal or a 45° turned grating next to a distractor. In this paradigm, the distractor stimulus was 90° clockwise turned compared to the target stimulus (Figure 2.11).

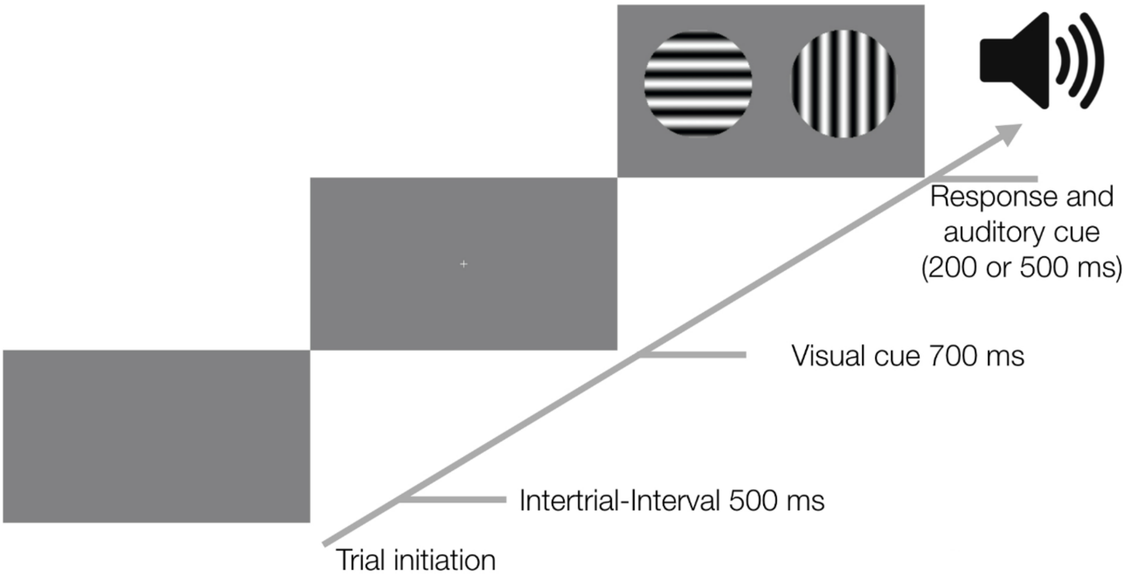


Figure 2.11: Stimulus presentation with a cardinal target from the script "VD cardinal.py" *The trial is started by an intertrial-interval of 500 ms, followed by a visual cue for 700 ms. Afterward, the stimuli are presented. In this paradigm, the horizontally orientated sine-wave grating is the target, while the vertically orientated sine-wave grating is the distractor. According to the response, the auditory cue is short (200 ms) or long (500 ms)*

2.3.6. Staircase Procedure

The staircase procedure in an orientation discrimination task is one way to measure orientation discrimination thresholds within one session. The stimulus presentation starts at the maximum orientation difference of 90 °. Every correct response by the mouse leads to an increase of difficulty by decreasing the orientation difference by 3°. A wrong response decreases the level of difficulty by increasing the orientation difference by 8° (Kaernbach 1991, see Eq. 4 to Eq. 6). The change in orientation differences over one experimental session as well as the identified turning points is shown in Figure 2.12. The mean of all turning points is the orientation difference at the desired performance.

$$Steps\ up * p = Steps\ down * (1 - p) \qquad (4)$$

$$p = \frac{1}{(1 + \frac{Steps\ up}{Steps\ down})} \qquad (5)$$

$$0.727\ \% = \frac{1}{(1+\frac{3}{8})} \qquad (6)$$

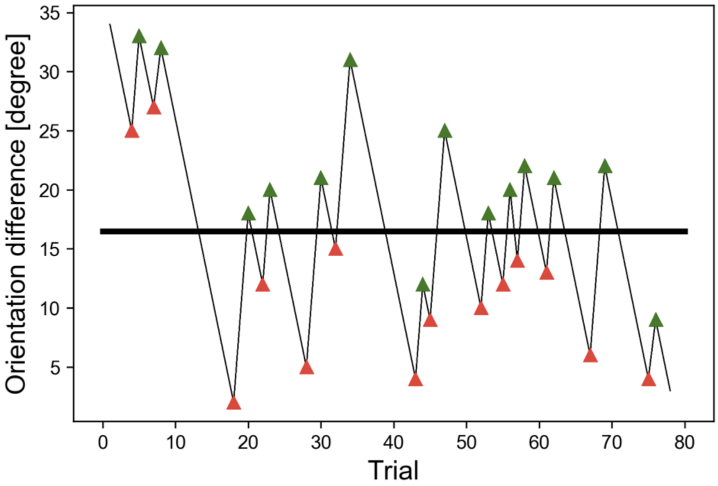


Figure 2.12: Example trials from a session with a weighted staircase procedure *The decrease in orientation difference indicates correct responses, while an increase is followed by an incorrect response to adapt the difficulty of the task. The green triangles are local maxima and indicate a wrong answer after a sequence of correct answers. The red triangles are local minima and indicate are correct answer after a sequence of wrong responses*

2.3.7. Bias correction

The bias correction detects two distinct strategies in animal behavior. On the one hand, it is detected, when the animal has a bias to the right or left side. On the other hand, it also detects if the animal is answering more often to the same side as in the trial before or if it is using an alternating strategy. Besides the detection, the stimulus presentation is determined by the strategy. Therefore, the last ten trials are analyzed with respect to the strategy the animal is using. If a strategy is detected, the algorithm counters this strategy, e.g. if the animal is always responding to the left side, the target stimulus will be presented on the right side. S corresponds to the count of trials, where the animal responded to the same side as in the trial before. O stands for the opposite side, while R represents the right side and L the left side, respectively. The bias correction corresponded to the following equation (Knutsen et al. 2006):

$$\begin{aligned} |S - O| > |R - L| &\rightarrow \begin{cases} S > O \rightarrow O \\ O > S \rightarrow S \end{cases} \\ |S - O| < |R - L| &\rightarrow \begin{cases} R > L \rightarrow L \\ L > R \rightarrow R \end{cases} \qquad (7) \\ |S - O| = |R - L| &\rightarrow \ U\,([L\ ,\ R]) \end{aligned}$$

2.3.8. Position tracking

The position tracking was done by a deep learning algorithm called DeepLabCut. DeepLabCut is a python-based toolbox for motion tracking in behavioral experiments. The structures, which had to be tracked were marked within a graphical user interface to create a training dataset. After the training, the artificial neuronal network was used to analyze all videos of the same kind. A new network has to be trained if the videos to analyze are changed in terms of their parameters, e.g. light conditions, the color of the mouse, contrast.

2.3.9. Statistical Analysis

The statistical analysis is performed with the SciPy-package for python. Different delay timings were tested with the Kruskal-Wallis test. Single sessions were tested for bias with a binomial test (p=0.5, n=number of trials). The experimental data was stored in individual csv-files for every mouse and sessions. The readout of these files was realized with a python script using the np.genfromtxt()-function from the NumPy-package.

2.4. Results

2.4.1. Training data

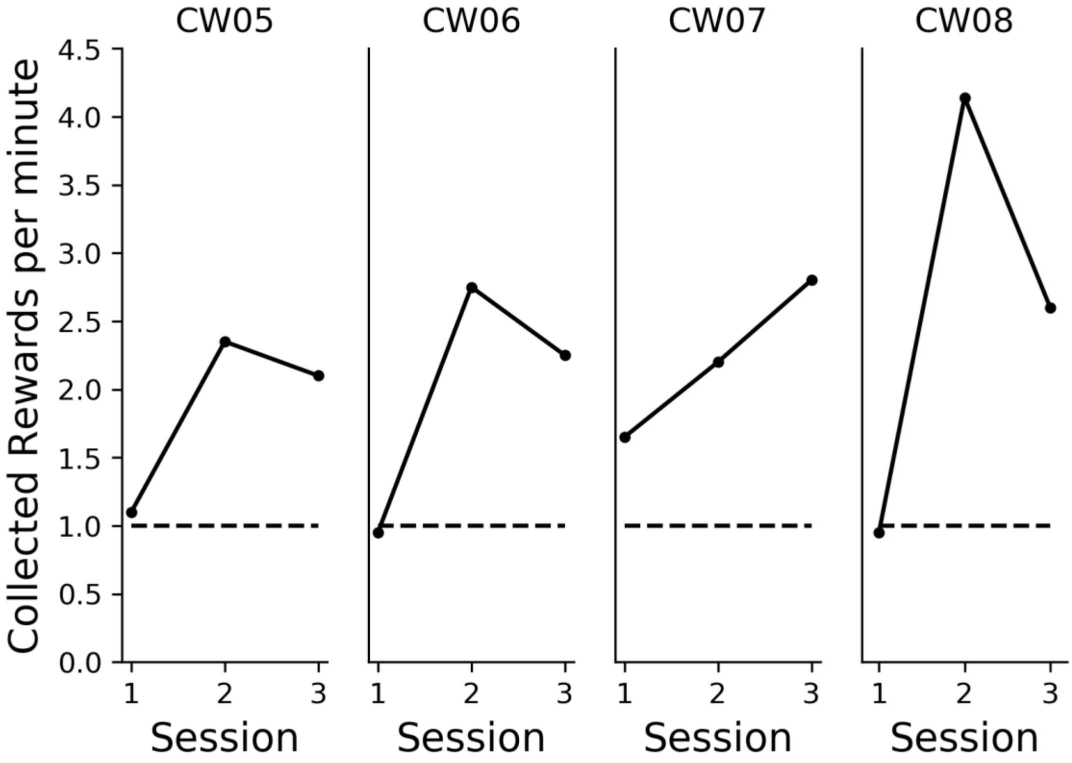


Figure 2.13: Licks per minute in the habituation phase of four male mice. *Every trace represents the collected rewards per minute by an individual mouse. To collect a reward the mice had to touch the water spout when the green light is activated. The black dashed line indicates the threshold, which is needed to be passed on two consecutive days to surpass the next stage of pretraining. One session was performed per day.*

The first step in the pretraining procedure leads to a certain level of habituation. Since the animals are already water-deprived at this time, they easily learn to receive water in the touchscreen chamber. Therefore, the animal can be placed into the touchscreen chamber for 20 minutes. Every time the green LED is turned on, the lick detection is activated and the animal can collect a water reward. Every delivery of a reward is followed by a timeout of five seconds. The criterion to pass this stage of pretraining is one lick per minute. Two animals started below the criterion, while the other two animals already collected a water reward more frequently than once per minute (Figure 2.13). In the second session, all animals showed a steep increase in licks per minute to collect a reward. They were all above the criterion in the second session. In the third session, they remained the level above the criterion, but three out of four mice showed a decline in licks per minute. Only mouse CW07 showed a linear increase in licks per minute throughout the three conducted sessions. Nevertheless, they all surpassed the criterion after two or three sessions.

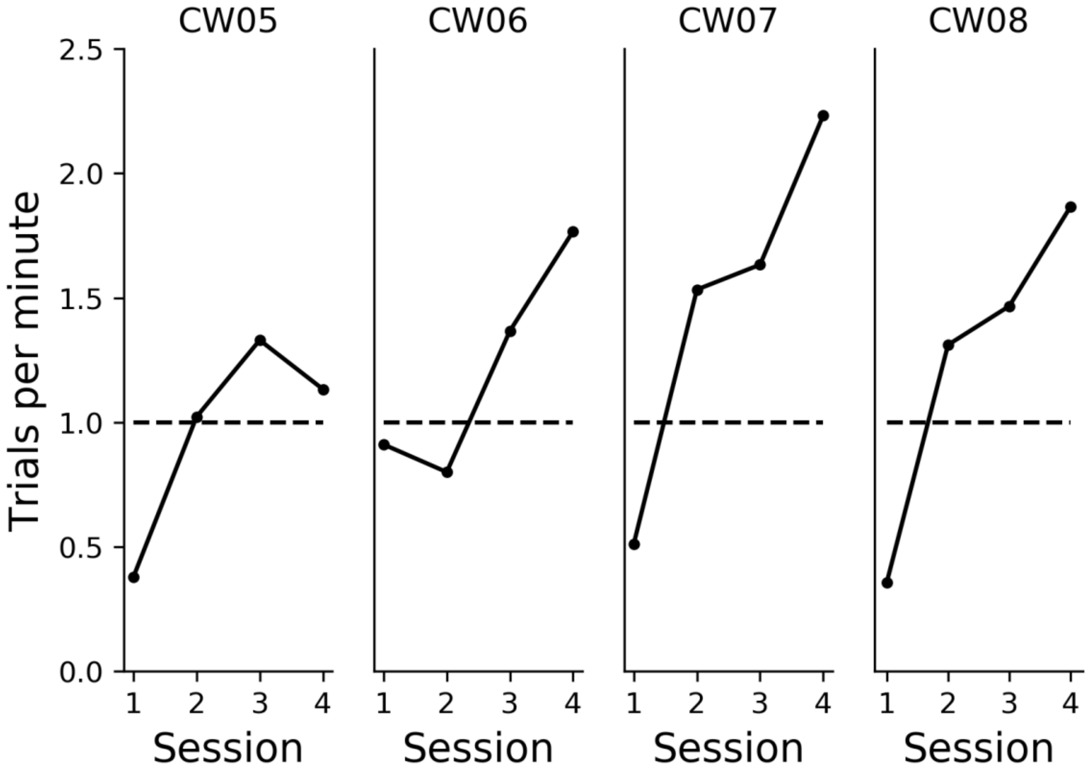


Figure 2.14: Licks per minute while the animals had to learn to touch the screen in order to get a reward *Every trace represents the trials per minute, which a mouse completed over several sessions (four mice). One session per day was performed. The black dashed line indicates the threshold, which is needed to be passed in order to proceed to the experimental training.*

Phase 1 of the pretraining has the goal to teach the animals to touch the screen in order to get a water reward (Figure 2.14). The single sessions had a different duration since it took some time until the animal figured out to touch the screen in the first session. Therefore, 45 minutes per animal were planned for the first session. Normally, the first touches on the screen happen unintentionally since there is no further priming. Due to that fact, there is a large variety regarding the performance of animals, after the start of this pretraining phase. In their respective second session, which lasted for ca. 30 minutes, three out of four animals reached the criterion for the first time. The third and fourth sessions lasted 20 minutes, each. Overall, the performance of the animals was rising.

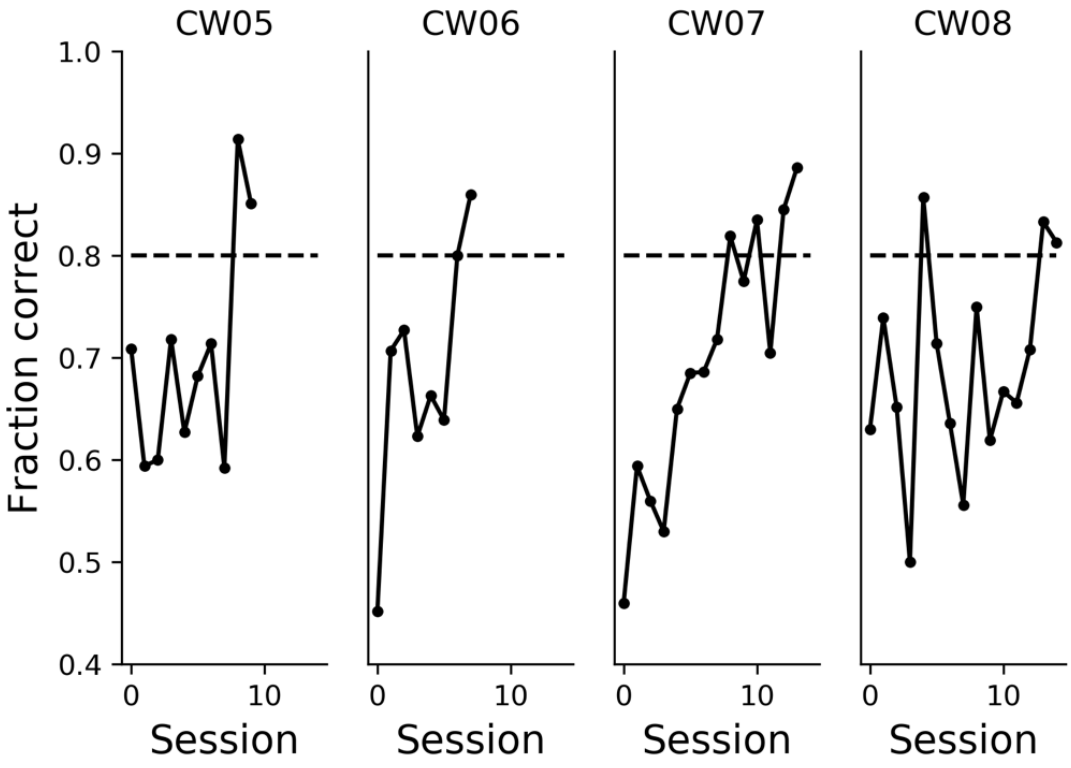


Figure 2.15: Learning curves of four different male mice. *The fraction correct over sessions in the visual discrimination training is shown for four different male mice. Every trace represents one mouse. One session was performed on one day. The black dashed line indicates the threshold of 80 % correct which is needed to be passed on two consecutive days, to consider that the mouse learned the task*

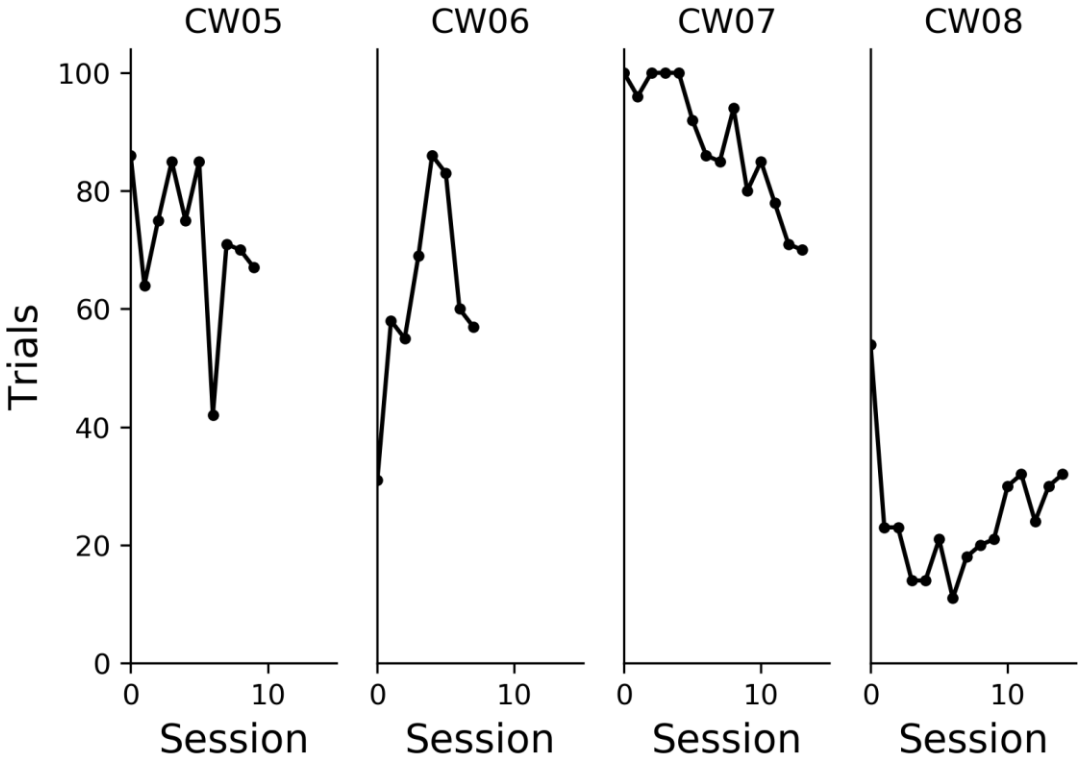


Figure 2.16: Number of trials in the visual discrimination training over four different male mice *As a marker for the motivation the number of trials was tracked. The black line indicates the number of trials performed by each animal per session. Every session was performed on an individual day.*

After each of the four mice completed the pretraining, the experimental training on the 2AFC-task was introduced. CW05 started with a performance above 70% and 86 trials in the first session. In the following sessions, the performance decreased to 60% and increased again in the fourth session. The performance is stable until a drop of more than 10% occurs in the seventh session. In this session, the mouse also performed a strikingly lower number of trials. After this drop, CW05 increased the number of trials again and also reached a performance above 80%. Overall it took 10 sessions to pass the criterion of 80% on two consecutive days.

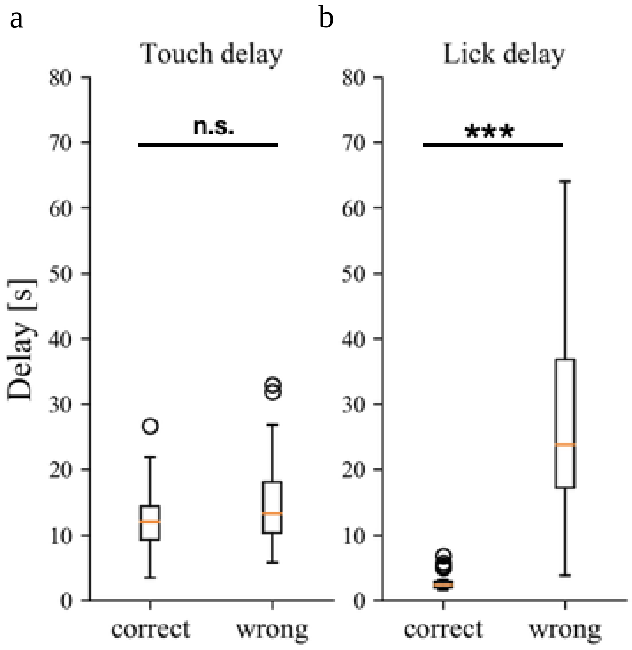
CW06 started with a performance below the guess rate (45%) at a number of 31 trials. On the following days, the mouse was able to increase the performance to 71% and 73%, while the number of trials was increased to 58 and 55. In the fourth session, the mouse increased the number of trials to 86 while showing a performance of around 65%. In the seventh session, an increase in performance up to 80% was observed. In the eighth session mouse, CW06 passed the criterion.

Mouse CW07 started with the highest amount of trials within a session and remained on a level with more than 80 trials per session. The learning curve started on guess rate-level and increased stable over time. In the ninth session, the animal passed the 80% performance level for the first time. In the following session, the performance decreased below the 80% threshold. Repeatedly, a session later the mouse passed the 80% threshold but dropped below again on a subsequent day. In sessions 13 and 14 the animal stabilized the performance above 80 %.

CW08 had, except in the first session, consistently the lowest number of trials. The performance of CW08 was very variable. In session 4, the mouse passed the 80% threshold for the first time, followed by nine sessions, where the criterion was not reached. CW08 reached the criterion in session 14 and 15.

Overall it was observed that the animals reached a performance of 80 % on two consecutive days after 8 to 15 sessions (Figure 2.15 and Figure 2.16).

2.4.2. Touch and Lick behavior



N=6 mice, 46 sessions,
2429 trials

Figure 2.17: Touch and lick delay in correct and wrong trials

a) The delay between the initiation of a trial and the response of the animal shown as boxplots. The delay is divided into correct and wrong responses. There was no statistical difference.

b) The delay between the response to a stimulus and the touch of the lick detection divided into correct and wrong trials. The lick detection was significantly faster activated in correct trials, which shows that the feedback is clear to the animal.

During an experiment, it is possible to collect more data than pure performance. The touch delay after the stimulus onset or the lick delay, after the activation of the lick detection, can give valuable information, especially when experimental and control groups are compared. To provide a benchmark for wildtype mice, the delay times were pooled in Figure 2.17. The touch delay did not differ in terms of correct or wrong responses (Kruskal Wallis test=2.46; p=0.117). The response to the stimulus happened in most of the trials within 15 seconds. Opposing to that, the animals reacted differently to the feedback they received when they responded correct or wrong (Kruskal Wallis test=67.23; $p=2.42*10^{-16}$). If they responded correctly, they collected their reward within a few seconds, while the response to negative feedback was followed by a short period of inactivity. The investigation of this inactivity period also led to the assumption that an animal, which stays inactive for more than two minutes does not have a temporal lack of motivation due to negative feedback. This justifies the abortion of the experiment after two minutes of inactivity.

2.4.3. Bias

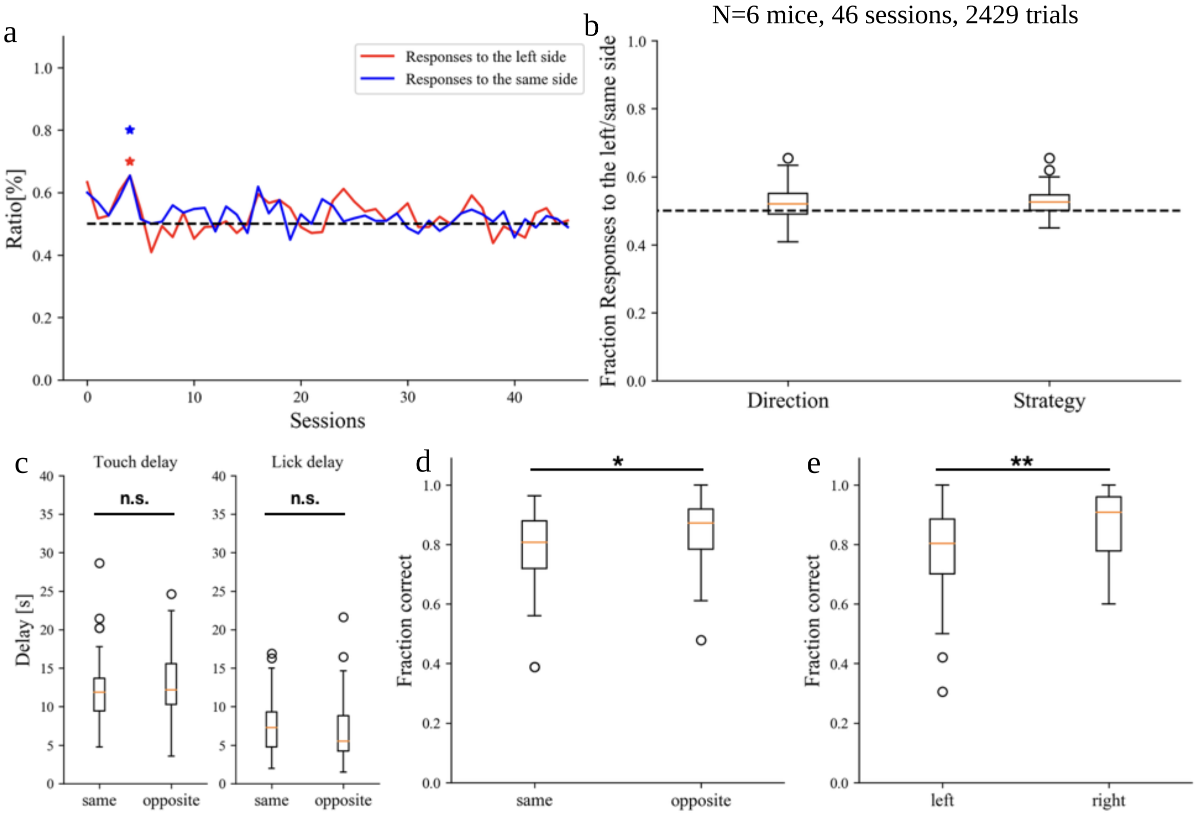


Figure 2.18: Bias analysis from a behavioral experiment performed in six mice.

a) Ratio of responses to the left (indicated by the red line) and the same side (indicated by the blue line) over individual sessions.

b) Pooled ratios of responses to the left or the same side over six mice and all performed sessions.

c) Comparison of the touch and lick delay in trials where animals picked the same or the opposite side as in the trial before.

d) Comparison of the fraction of correct trials for trials, where animals responded to the same or the opposite side compared to the trial before.

e) Comparison of the fraction of correct trials for trials, where animals responded to the left or the right side

The direction bias could not be shown in this experimental dataset. As shown in Figure 2.18(a) for an experiment with six animals and more than forty sessions, there is only one session which had a significant bias regarding the strategy of choosing always the same side as in the trial before (tested with binomial test). The red line indicates the responses to the left side and the blue line the responses to the same side as before. The asterisks above indicate a significant bias. The dashed line indicates the rate, where no bias is present. Overall there is the trend, that the animals are a bit more likely to answer to the left side and to the same side as in the trial before. It has to be mentioned, that in the median, there is a slight trend to the strategy to respond to the stimulus position, which was chosen before, as shown in Figure 2.18 (b). Again, the dashed line indicates the rate, where no bias is present. This is not a significant trend. In conclusion, less than 5 % of sessions had to be taken into account as being biased. The delay times did not differ between responses to the same or opposite side as in the trial before (Kruskal Wallis test=1.78; p=0.18) as well as the lick delay did not show a difference (Kruskal Wallis test=1.48; p=0.223) shown in Figure 2.18 (c).

The choice of a strategy also affects the performance as shown in Figure 2.18 (d). The figure shows the performance level if the animal responded to the same or the opposite side as in the trial before (Kruskal Wallis test=4.06; p=0.04). If the correct stimulus in on the same side as in the trial before, a correct rate of 80 % was reached. In contrast to that, if the animal goes for the opposite side as in the trial before, it is more likely that the response is correct. This has to be compared with the bias correction. As shown before, the animals have a slight overall bias to respond to the same side. The bias correction works against that trend which leads to the fact, that it is advantageous for the animal to switch the side. Vice versa, animals who are responding alternating to the right and the left stimulus would benefit from responding to the same side as before. The changes in performance according to the strategy are indicating that the bias correction is actively acting against individual strategies. This results in different performance levels for the left and the right stimulus as well (Figure 2.18(e), Kruskal Wallis test=11.85, p=0.0006).

2.4.4. Position tracking

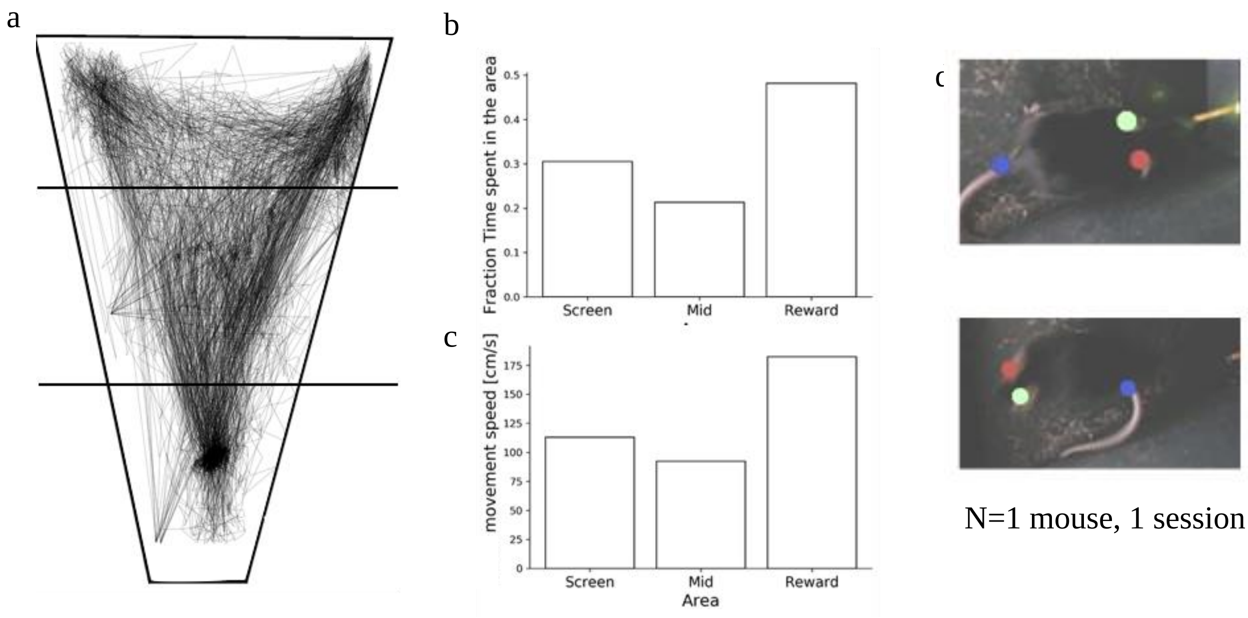


Figure 2.19: Example session of analyzed position data via DeepLabCut in one expert mouse

a) Raw trace of the mouse' trajectory in the animals' area of the touchscreen chamber. The touchscreen chamber is divided into three areas. Outgoing from the distance between the screen and the wall, where the water spout is attached, the first third is named as screen-area. The second third is the mid area and the last third is the reward area.

b) Time spent and mean movement speed in different areas. The barplots show the fraction of time an animal spent in every area of the touchscreen chamber after it gained the expert level.

c) The average movement speed in cm/s is shown.

d) Example frame from the DeepLabCut position tracking. Therefore, different structures were marked. The marked structures are the left ear (bright green), the right ear (red) and the root of the tail (blue)

The position tracking with DeepLabCut showed that the mouse used the full area of the touchscreen chamber (Figure 2.19a). The spot at the water spout cannot be reached since the water spout restricts the free access to the last centimeters of the area. The area can be divided into three areas: the screen area, the mid area, and the reward area. Each of them is one-third of the distance between the screen and the wall behind the water spout (Figure 2.19b). Most of the time was spent in the reward area. The animal had to reach the water spout after every trial, so it is likely, that the animals spent there a significant part of the time. They also showed the highest movement speed in the reward area (Figure 2.19c). This was also likely since the animals needed to reach the water spout in order to collect a reward or initialize the next trial. They spend the least time in the middle of the touchscreen chamber. After the training, the screen and the reward spout were meaningful areas for the animal. The mid-area was more used in the case of inactivity periods. 30% of the time the animals were in front of the screen. This can be assumed as the time they actually spent with the task to discriminate two different images. Figure 2.19d shows the marked structures. For reliable tracking, the two ears and the base of the tail are marked.

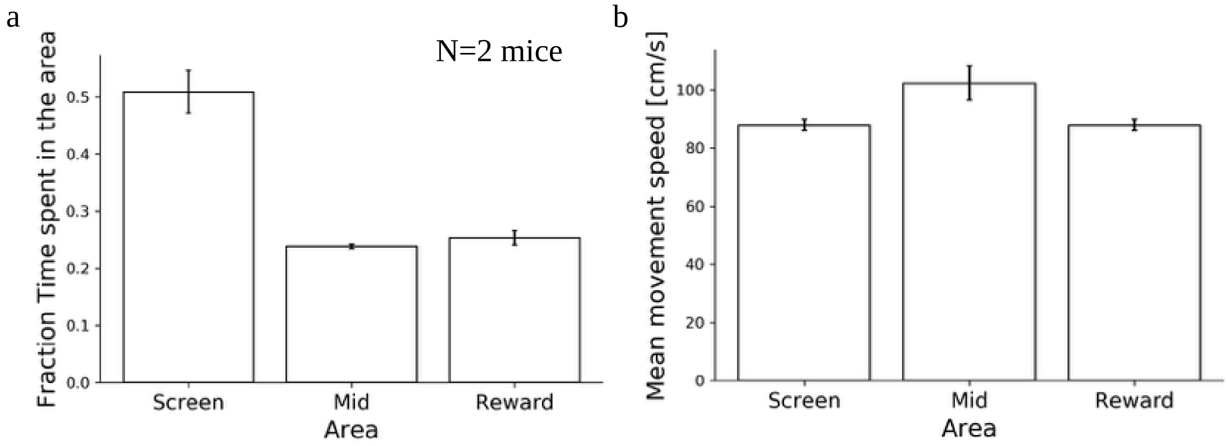


Figure 2.20: Example analysis of position data via DeepLabCut in two naive mouse

a) Mean Time (±SEM) spent in different areas. Analog to the previous figure, the three-thirds of the touchscreen chamber are divided into a screen-area, a mid-area and a reward-area. Most time was spent close to the screen, while there was less time spent in the mid- and reward area.

b) Mean movement speed (±SEM) in different areas for a naive mouse. There was no difference in the average movement speed per area in naïve mice.

Two naive animals showed a different behavior. They spent most of their time in front of the screen, while they spent less time in the mid area and the reward area (Figure 2.20a). They also showed the highest movement speed in the mid area, while the difference in the movement speed was less high (Figure 2.20b). The difference between naive and trained animals can indicate an actual learning process for the animals or additional differences between experimental and control groups, respectively, depending on the experimental design. This example is an illustration of the use of DeepLabCut in the context of the touchscreen chamber and does not answer a specific scientific question.

2.4.5. Summary

- The touchscreen chamber provides a basic flexible framework for a Two alternative forced-choice task but is not limited to it
- The experimental scripts are freely available and open-source
- The touchscreen chamber meets the animal welfare-requirements
- The touchscreen chamber is a viable tool to perform operant conditioning in mice within a reasonable time window
- The touchscreen chamber can reveal differences in the positioning pattern between naïve and trained animals
- The bias correction is acting against individual strategies by the animals
- The touchscreen chamber provides a comprehensive framework for behavioral phenotype screening in a wide range of different experimental paradigms
- The presented touchscreen chamber costs around 699 €

2.5. Discussion

Our proposed touchscreen chamber provides a comprehensive framework for operant conditioning of mice. We showed a comparable performance of the mice to other approaches in the field of animal training. All scripts are freely available and ready-to-use. This makes our touchscreen chamber a flexible tool, which can be used in several environments with different tasks. Here, we show the training from a naïve to an expert mouse including all pretraining steps. As additional functions we implemented a bias correction and demonstrated, that it worked as supposed. In addition we linked the touchscreen chamber with DeepLabCut to perform a proper position tracking of the mouse. All parts and software-solutions are freely available for neuroscientific labs.

2.5.1. Hardware

The presented touchscreen chamber combines the high demand for automatized, standardized and flexible operant conditioning in animals (Figure 2.6 – Figure 2.10). In comparison to other published approaches, the here presented operant conditioning chamber has a rather high cost. These approaches were delivered with limited functionality. All shown operant conditioning chambers, except the Bussey-Saksida Touchscreen Chamber are limited to the use with rats since the capacitive screen technology does not have sufficient sensitivity to the paw or nose of a mouse. The limiting factor here was the availability of infrared touchscreen frames of the correct size on the European market. The presented touchscreen chamber was built with an infrared touchscreen frame from China. From the perspective of a group that is located in Europe, it has to be considered, that it consumes more time if an infrared touchscreen frame needs to be changed. Therefore, it can be recommended that a spare infrared touchscreen frame should be available at all times. Despite that all hardware can easily be acquired by labs in different countries to ensure similar hardware standards.

Despite the infrared touchscreen frame, all materials of the presented touchscreen chamber are available on the European market within a few days and they are easy to assemble or disassemble without deeper technical knowledge.

2.5.2. Software

The software was approved in several experiments, which are described in the following chapters. Python, as a freely usable programming language, is a great environment for the experiments and the analysis, because it is not necessary to establish experimental pipelines across different platforms. The used and necessary packages are open-source and freely available as well. The software provides profound flexibility in terms of stimulus presentation. Besides the presentation of Gabor patch gratings, which are a built-in function of PsychoPy, it is also possible to present preprocessed frames or movies with PsychoPy. A big advantage of the use of Python and specifically PsychoPy is the possibility to realize different experimental paradigms. In the following chapters, a paired visual discrimination learning task and a staircase procedure for an orientation discrimination task is introduced. It is also possible to implement a 3AFC task or attention tasks. These paradigms are based on the 2AFC-visual discrimination task introduced before. Besides that, PsychoPy gives also non-programmers the opportunity to build desired paradigms. Therefore, it is possible to implement multimodal stimulation. In the presented task, the auditory cue is given as a feedback to the animal, but it can also be a part of decision-relevant stimulation.

2.5.3. Animal Training

The learning curves of the animals is comparable to other approaches, even though it is quite misleading to compare the learning curves of different experimental paradigms (Figure 2.13 – Figure 2.16). The most similar experiment by Yu et al. 2019 with the goal to train animals on the discrimination of natural scenes, showed a comparable number of needed sessions for the animals to understand that they get rewarded and that they had to touch the screen. On the other hand, the authors claimed that animals reached a criterion for the discrimination task within 5 to 15 days with another set of stimuli. It is not clear if their stimuli are comparable to two different orientated sine-wave gratings. Another task, designed by Andermann et al. 2010 showed a similar training procedure, where the animals had to learn orientation discrimination in a head-fixed task. Substituting the additional time for surgeries and handling, the animals took between two weeks and two months to complete the task with stable performance levels. It has to be mentioned that this is not perfectly comparable since the experimental setting differs due to the fact that the head restriction is a more stressful procedure for the mouse (Schwarz et al. 2010). A possible disadvantage that there is a dedicated operant conditioning chamber opposite to do the training in the homecage as in other presented approaches (Pineno 2013, O’leary et al. 2018, Leising et al. 2013) cannot be confirmed by the learning performance.

2.5.4. Touch and lick behavior

The animals did not show a difference in the delay to respond to the stimulus depending on if the response is correct or wrong (Figure 2.17). Thus, it is not possible to estimate the outcome of a trial by the time, the mouse needed to respond to the stimuli. Lempert et al. 2015 defined the reaction time to a stimulus as the response confidence. In the experimental training, the animals seemed to have, either in correct or wrong responses , the same level of confidence. As a proof-of-principle the delay to collect the reward after the response to the stimulus was recorded. The mice took a longer time to collect the reward when they responded incorrectly to the stimuli. That shows that the feedback in the form of a high-pitched tone and the bright screen is clearly understood by the animal. Clear feedback to the animal is fundamental for successful operant conditioning.

2.5.5. Bias

The bias correction proofed to be a helpful tool to correct the usage of strategies (Figure 2.18). Averaged over all sessions, the animals showed the tendency to respond to one side. This also comes with the fact, that the animals were more likely to respond to the same side as in the trial before. The reaction time to the stimulus or the lick delay to collect the reward was not affected by strategy. Nevertheless, the performance was significantly increased when the animals act against their detected direction or strategy bias. Pruskey et al. 2000 identified three different possible strategies, a side preference, an alternating response pattern or the animals stay on one side, when they were rewarded on this side in the trial before. The used bias detection corrects for side preference and alternating strategies. A strong effect of a reward-dependent reward cannot be derived from the shown data. The bias correction is necessary, which is also shown by the differences in the performance level. In case there is absolutely no bias, a difference was not expected in the performed analysis. By the fact, that there are slight differences, we can show the strong evidence, that the bias correction was active and therefore necessary. Other studies in mice (Yu et al. 2018) showed that their mice did mainly show no side preference at all. The presented results lead to another conclusion.

2.5.6. Position tracking

The addition of the Deeplabcut-Framework is an extremely helpful tool when the scientific question is related to the exact movement pattern of the animal (Figure 2.19).The analysis showed, that it could be possible to reveal differences between different mouse groups. In this thesis, the number of analyzed videos was too low, to draw conclusions for naïve and expert mice, but if this technique fits a specific hypothesis, it is possible to link the touchscreen chamber with the Deeplabcut-framework. . Different task solving strategies, e.g. where the animal made a decision, can be revealed with the position tracking. The Deeplabcut-framework is not directly implemented into the software scripts used for the touchscreen chamber, so a live tracking is not possible. If newer versions of Deeplabcut provides the functionality for live tracking, it is possible as well to couple it tightly to the experiment, e.g. with auditory cues if the animal shows a period of inactivity.

2.5.7. FAIR principles

The data output follows the FAIR principles for the storage of data. The csv-format, in which the behavioral data is saved, is highly flexible. It is possible to find a csv-file for every session, marked with the mouse id, the date and the experimental script. In the future this simple structure can also be integrated in an SQL-database. The data is highly accessible and readable by humans and machines. All analysis in this thesis was made based on python-scripts, but the format is also compatible to Microsoft Excel or MatLab. For every session a metadata-file is saved as well. Another advantage of csv-files is the low needed space on harddrives. Therefore, the data can be made re-operable and transferable with minor changes. This is a big advantage with view on reproducibility as well. The biggest problem for reproducibility was described to be a insufficient reporting of the data. With the presented solutions, all needed data is saved into one csv-file and one metadata file. It can be argued if another description of the task would be helpful, but here we present a comprehensive data output which should enable the reproduction of an experiment.

2.5.8. Animal welfare

The procedure is at least in line with two of the three 3R-principles. A complete replacement of animals is not possible if such behavior is investigated. By now, it is not possible to replace a behaving animal. The demand for reduction of animals needed for experiments is given due to the high level of standardization and the possibility to repeat experiments over and over again with individual animals. Due to our experience, animals perform until the age of above a year stable in different tasks. Also because of standardization and automatization, the requirement for refinement is fulfilled.

2.5.9. Conclusion

Here we present a comprehensive solution for operant conditioning in rodents, flexible enough for the use in other species. The touchscreen chamber provides a software framework, able to build different experimental paradigms. All parts of the touchscreen chamber are simple to assemble and are available by various vendors. We showed the training data of four different animals as well as additional recorded parameters. The animals learned the task after the pretraining within eight to fifteen sessions and is able to compete with commercial solutions like the Bussey-Saksida touchscreen chamber. In addition, the presented touchscreen chamber provides a higher functionality than the presented other in-house solutions for operant conditioning. According to the scientific question as well as the experimental design, a various number of measurements can be recorded. Here we showed analysis of the reaction time to the stimulus and the reward delivery system, as well as position tracking and bias analysis. As a proof-of-principle the position tracking is viable to measure the speed of the mouse and movement patterns on a trial-to-trial basis. The bias correction turned out be a vital feature, since it is possible to detect slightest preferences in the animal and correct for this factor. The method can be used in the same way in different laboratories with different paradigms, so we also provide a comprehensive approach with view on reproducibility.

Chapter 3: The functional role of neuronal overrepresentation of cardinal orientations

3.1. Abstract

Orientation tuning in the primary visual cortex and other brain areas is a well-described phenomenon. The tuning of these neurons is varying over different orientations. It has been shown that the cardinal orientations are overrepresented over oblique orientations in the neuronal responses, they evoke. Other studies also show the behavioral effects of these tuning properties in various species, but there is a lack of data for mice. Even orientation discrimination tasks are an often-used paradigm in the research for the mouse visual system, the functional effect of the overrepresentation of neurons tuned for cardinal orientations is not yet described. Therefore, the training procedure should be considered when investigating sensory discrimination capabilities of the animal. By using a python-based touchscreen chamber, we wanted to close this gap. Firstly, a parallel visual discrimination task was introduced. Two distinct sets of stimuli, one with the discrimination between a horizontal and a vertical grating and one with two oblique orientated gratings at a difference of 90°, were shown randomly. The animals learned the discrimination between the horizontal and vertical orientated gratings faster. In addition, a Two-Alternative-Forced choice task was performed, where the animals had to distinguish between an orientation-fixed target and a variable distractor. In an adaptive staircase procedure, the specific psychophysical discrimination thresholds can be measured. We used two different paradigms: In the first place, animals were overtrained to distinguish between a horizontal and a vertical orientated sine-wave grating. Afterward, they were retrained to distinguish between an oblique orientated target and a cardinal orientated distractor. After they reached a comparable performance, the staircase procedure with an oblique target was reintroduced. The animals showed a large decrease in orientation discrimination performance. In the second setting, the mice were trained on an oblique target from the start. They showed lower orientation discrimination thresholds, which are comparable to the thresholds they achieve for a cardinal target. Thus, we can conclude, that there is a functional consequence of cortical orientation tuning, but this effect can be decreased with specific training. This may argue for a plastic change in the tuning properties of sensory neurons and therefore a bias the discrimination performance.

3.2. Introduction

3.2.1. Orientation tuning in the primary visual cortex

The first evidence for cortical orientation tuning in mammals was found in the cat´s striate cortex (Hubel & Wiesel 1959). Later, similar findings for spider monkeys were published (Hubel & Wiesel 1960). Cats and monkeys are historically the dominant species in the research of the visual system but there is also ongoing research in the mouse. Due to progress in different imaging approaches and their genetic characteristics mice became more and more a prominent face in the research of the visual system (Huberman & Niell 2011). Research in the mouse primary visual cortex also showed the presence of orientation-selective neurons. There were more neurons selective for cardinal orientations than for oblique orientations (Roth et al. 2012). How strong they are tuned to a certain direction can differ. This is derived from the circular variance, a measurement which quantifies the response of a neuron to a certain orientation. If a neuron responds to all orientations equally, the circular variance is equal to 1, which is corresponding to an orientation selectivity of 0. If a neuron is only responding to one orientation, the circular variance is equal to 0, corresponding to an orientation selectivity of 1 (Ringach et al. 2002). Most of the excitatory neurons show an orientation selectivity around 0.4, while the inhibitory neurons are less strongly orientation-selective (Kerlin et al. 2011, Sohya et al. 2007). There is no significant difference between the types of inhibitory neurons. They all show an orientation selectivity between 0.1 and 0.2 on average (Figure 3.1).

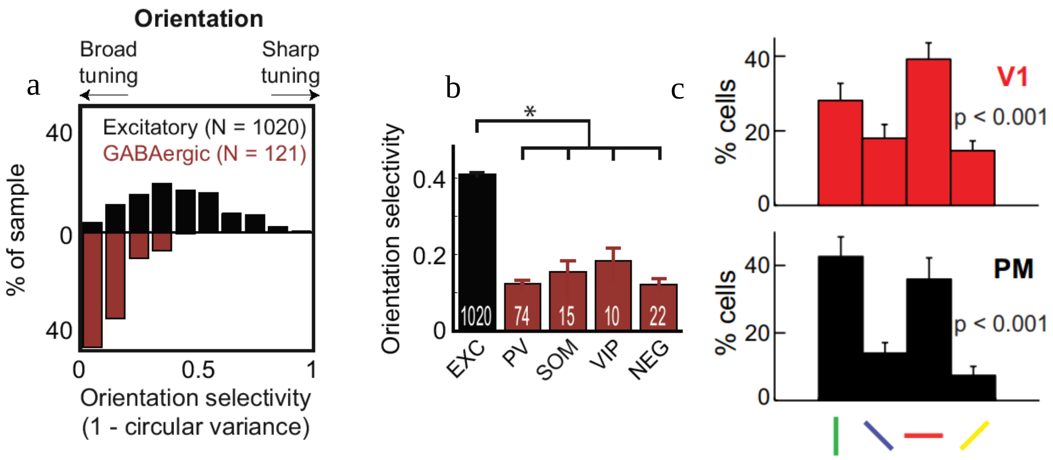


Figure 3.1: Orientation selectivity in excitatory and GABAergic cells of the primary visual cortex (from Kerlin et al. 2010)

a) The distribution of orientation selectivity in excitatory cells of V1 (black) and inhibitory GABAergic cells (red).

b) The average orientation selectivity of excitatory (EXC), parvalbumin (PV), somatostatin (SOM), vasoactive intestinal peptide (VIP) and immunohistochemically negative (NEG) neurons

c) The distribution of orientation selective neurons in V1 and PM (Roth et al. 2012)

The computation of orientations is quite crucial for a visual system. Orientations are found on the edges of visually perceived structures and therefore, they are important for the perception and processing of the image information (Maini & Aggarwal 2009).

The neurons are not uniformly selective to all present orientations. There are more neurons, which are selective for cardinal orientations than for oblique orientations, shown with electrophysiological recordings and calcium imaging (Metin 1988, Roth et al. 2012, Atallah et al. 2012). The cardinal overrepresentation is also seen in the image statistics of natural images, where fewer oblique orientations are found (Girshick et al. 2011, Figure 3.2). This leads to the question, if there is a functional difference in the perception and discrimination of these distinct types of orientations.

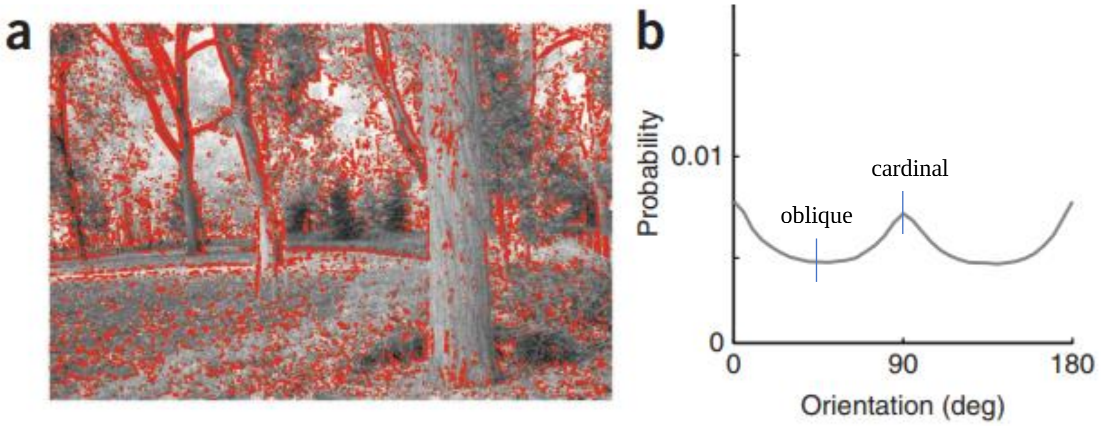


Figure 3.2:Orientation statistics in natural images (adapted from Girshick et al. 2011)

a) Example photograph of a natural scenery with red marked edges to illustrate different orientation information

b) Distribution of the orientations over a database of natural images including 654 images from the McGill Database

3.2.2. Orientation Discrimination

As mentioned in the previous subchapter, the perception of edges and their orientation is very important to actually recognize the environment in a proper way. Orientation discrimination is an ongoing field of research in understanding the cortical perception of visual stimuli. The first recorded orientation discrimination in freely-behaving mice was done by Reuter 1987, who trained mice to distinguish between a horizontal target and a vertical distractor. Therefore, the mice were placed in a starting box, which had two doors. One door led into another box with a reward, the other door led to a box with an apparatus to provide a foot shock. The door to the reward box was marked with a horizontal orientated bar pattern, equal to a square wave grating, while the punishment box was marked with a vertical pattern.

Another approach to train mice without a head-restriction is the visual water maze task (Prusky et al. 2000). In this setup, the link between orientation discrimination contrast and spatial frequency was shown (Prusky & Douglas 2004). In a behavior task with head-restrained mice, where they had to lick a water spout whenever a horizontal orientated square-wave grating was presented, the mice reached a discriminability of 1.7 at 11°. Therefore, different orientated gratings were shown in a Go-NoGo task. Thus, the animals also had the possibility to leave out a reaction to the stimulus. The highest discriminability in this task was shown for an orientation difference of 45° (Anderman et al. 2010). All presented experiments aimed for a horizontal or a vertical target. Little is known about the behavior of the mouse when it comes to an oblique orientated target.

3.2.3. Cardinal dominance in visual orientation discrimination

When it comes to the perception of oblique orientations there is much evidence for an oblique effect in other species than mice. In humans, the first evidence for an oblique effect was shown with respect to the visual acuity (Emsley 1925). In this experiment about the effect of correcting lenses, it was shown that there is a decrease in the visual acuity when oblique orientations are shown. It is possible to attenuate this effect by tilting the head (Higgins & Stultz 1950). Another strong evidence for a so-called oblique effect in humans is the fact that wires or lines are easier to detect when they are orientated horizontally or vertically (Ogilivie & Taylor 1958, Ogilivie & Taylor 1959). In other species, the presence of an oblique effect was described as well.

As one of the closest relatives to the mouse, rats are slower in learning the discrimination between oblique orientations than for cardinal orientations at a stable orientation difference (Lashley 1938). Therefore, the rat was placed on a platform in front of a wooden plate with a gap in between. Two cards, one with a horizontally orientated and one with vertically orientated square-wave grating like pattern. While the unrewarded stimulus was placed in front of the wooden plate, there was a space with a reward behind the rewarded stimulus. The rat had to jump to the stimulus. In case of a wrong response, the rat bumped against the wooden wall and fell down. This was reproduced in squirrels, who were not able to learn the discrimination between a horizontal target and an oblique distractor (Dodwell 1970). The effect, that oblique orientations are less likely to be learned in visual discrimination tasks is also shown in octopuses (Sutherland 1957), Pigeons (Zeigler & Schmerler 1965) and goldfish (Mackintosh and Sutherland 1963).

The functional relevance of the overrepresentation of cardinal neurons was also showed in humans (Girshick et al. 2011). In their setting they used a stimulus including a cloud of different orientated sine-wave gratings. The participant had to decide if the mean orientation is clockwise or counter-clockwise. They were able to show that if the mean orientation is close to a cardinal orientation, it was easier to make a correct choice as if the mean orientation was closer to an oblique orientation. They supported their findings with the analysis of natural images, which showed that cardinal orientations are overrepresented in natural environments as well.

3.2.4. Goal

Since the behavioral oblique effect, as well as the neuronal oblique effect, was shown in a wide range of studies (Metin 1988, Roth et al. 2012, Atallah et al. 2012), there is a gap for the behavioral oblique effect in mice. This chapter aims to close this gap and shows the behavioral oblique effect in mice. Therefore, the touchscreen chamber described in Chapter 2 is used. We wanted to show that mice are worse in learning an oblique target in a visual discrimination task compared to cardinal targets. To show this effect, we designed a parallel visual discrimination learning task, which presents both, cardinal and oblique targets, at equal difficulties, to distinguish. The studies, which showed the neuronal oblique effect delivered huge evidence that these tuning properties are also represented in the behavior. Therefore, we are also interested in the psychophysical thresholds in the discrimination of gratings of different orientations. Thus, we aim for establishing an adaptive staircase procedure, which was, to our knowledge, not done in the mouse visual system before. So, this experiment is also a proof-of-principle for the presented method from Chapter 2 to support the usability in a wider range of psychophysical experiments. In addition, we tested the for a plastic effect in different experimental training paradigms.

3.3. Methods

3.3.1. Animals

The parallel visual discrimination learning task was performed in four male C57BL/6 mice, started with the experiments at an age of eight weeks. The orientation discrimination experiment including the staircase procedure was performed with 12 male C57BL/6 mice at an age of 8 weeks at the beginning of the experiments. The experiments were authorized in the licenses 84-02.04.2016.A357 and 84-02.05.40.17.055. They were bred and kept at the animal facility of the institute (RWTH Aachen University). During the experiment, the animals were kept on a turned Day/Night-cycle of 12h:12h. The animals are water restricted through the experiment. They receive water *ad libitum* on one day of the week on which no experiments are performed. Per day they receive at least 1.5 ml of water to maintain their wellbeing. They have access to food *ad libitum* all the time. The mice are weighted and observed before the experiments starts. The animals are scored for deviant behavior to check for stress symptoms, injuries or other relevant symptoms. This includes the activity of the animal, the movement, the breathing frequency and skin turgor. Minor impairments are scored with one point, major impairments are scored with two points. If there are no major impairments observed, the animal has to be monitored carefully if the cumulative score is between one and four. At a cumulative score of 5 or higher or a major impairment the animal has to be removed from the experiment and has to be euthanized. At no point of the experiment a euthanasia was conducted.

3.3.2. Touchscreen Tasks

The touchscreen tasks were performed in the touchscreen chamber, described in Chapter 2. There were no changes to the final version of the touchscreen chamber.

3.3.3. Habituation

In this phase, the animals were supposed to habituate in the touchscreen chamber and link a green light from an LED with the availability of a reward. Therefore, the mouse is placed in the middle of the box and the training script is started. At this moment the green light turned on and the lick detection was activated. When the animal triggered the lick detection at the water spout, a water reward is delivered and the green light is turned off.

This was followed by a timeout of five seconds. This procedure lasted for 20 minutes to avoid stress in the mouse. At the end of the experiment, the number of reward deliveries is displayed. The criterion to proceed to the next phase is one response to the lick detection per minute on two consecutive days.

3.3.4. Learning to touch the screen

This phase consisted of the task to touch the screen in order to receive the reward. The mouse is placed in the box and the script can be started then. Initially, the screen is turned on and showed a generic vertical orientated sine-wave grating. A touch on the screen is followed by a low-pitched tone for auditory feedback. After the mouse touched the screen, the green light turns on which indicated, as learned in the phase before, that a reward was ready to get collected. On the first day of this phase, one session lasted 45 minutes, on the second day 30 minutes and from the third day on for 20 minutes. The program displayed the number of touches and reward collects at the end of the session. The number of touches, as well as the delay times, are saved to a csv-file. The criterion to proceed to the experimental training was one touch per minute.

3.3.5. Experimental training for orientation discrimination

After the mouse learned to touch the screen in order to get a reward, the animals were introduced to an easy orientation discrimination task. Therefore, two different orientated sine-wave gratings were shown with a difference of 90°. In this setting, the target was either horizontally orientated or at an oblique orientation of 45°. The distractor was turned 90° in the clockwise direction. The duration of a session depended on the motivation of an animal. After two minutes of inactivity, the sessions were stopped and the program created the results in a csv-output file. When the animal touched the correct stimulus, a low-pitched tone -equal to the low-pitched tone in the phase where the animals had to learn to touch the screen- and the green light turned on. The animal was now able to collect a water reward. The water reward is delivered by three short activations of 0.1 seconds. This gave an additional auditory cue due to the clicking of the valve. In case of a wrong response, the screen turned bright to give visual feedback. At the same time, a high-pitched tone was played. The mouse had to trigger the lick detection in order to initiate the next trial. An animal was considered to perform reliably when it reached a performance of 80 % on two consecutive days.

3.3.6. Retraining

After reliable learning of the discrimination between a horizontal and a vertical grating, the animals were trained on the discrimination between an oblique and vertical grating with a changed reward scheme. Previously, the cardinal grating was always the target. Now the animals had to learn that the oblique orientated grating was the target in the same visual discrimination task described in Chapter 2. The goal was to achieve the same performance at a difference of 45° for an oblique target as for a cardinal target. Afterward, the staircase procedure was performed with these animals to quantify their orientation discrimination threshold for an oblique target after retraining.

3.3.7. Staircase Procedure

After the animals learned the task reliably, they can proceed to the staircase procedure. The animal was placed in the touchscreen chamber and the program is started (see “staircase cardinal.py” and “staircase oblique.py”). The first orientation difference was chosen randomly between 0° and 90° to check if the initial orientation had an influence on the orientation discrimination threshold at the end of the session.

The consequence of correct and wrong responses was equal to the experimental training. A correct response was followed by a high-pitched tone, the onset of a green LED above the water spout and the activation of the lick detection, which enabled the water delivery as soon as the animal touched the water spout. A wrong response was followed by a bright screen and a high-pitched tone. In this case, the animal had to initiate the next trial with a touch of the water spout without getting a water reward delivered. When the animal responded correctly, the orientation difference in the next trial is decreased by 3°. In opposite to that, the orientation difference was increased by 8° in the next trial when the response is wrong. This was only applied when the actual orientation difference is not smaller than 5° for correct trials and not larger than 90° for wrong trials. This was implemented to avoid orientation differences below 0°, which results in a counter-clockwise turning or an orientation difference above 90°, which would result in an actual orientation difference below 90°, due to the fact that a grating which is turned by 180° have the same perceived orientation as 0°.

The analysis of the staircase procedure takes the turning points into account. Every trial is used to calculate a new orientation difference. Other approaches also use a block of trials (Gianfranceschi et al. 1999). Figure 3.3 shows an example for a session of the weighted staircase procedure, in specific how the orientation changes over the trials according to the animals’ behavior. The identified turning points are marked as well.

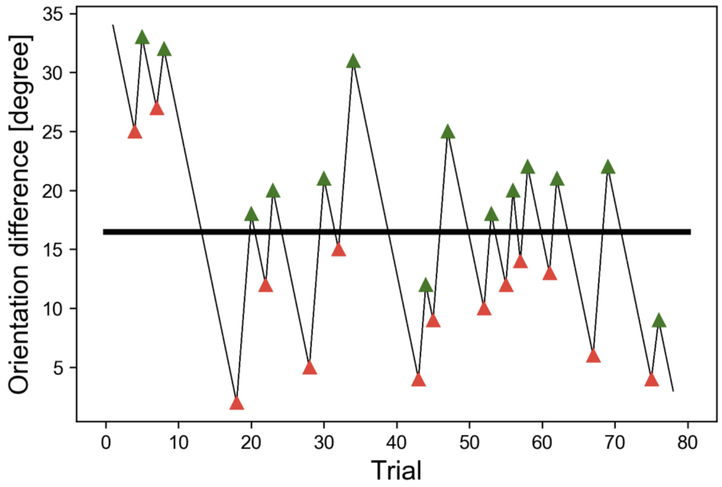


Figure 3.3: Example session with a weighted staircase procedure. *The black line indicates the orientation difference shown in every trial. An increase in orientation difference indicates incorrect responses by the mouse, while correct responses lead to a decrease of the orientation difference. The green triangles are local maxima and indicate a wrong answer after a sequence of correct answers. The red triangles are local minima and indicate are correct answer after a sequence of wrong responses*

The green triangles indicated a turning point after a number of wrong responses, while the red triangles indicated a turning point after a number of correct trials. The mean of all turning points is considered as the orientation discrimination threshold for this session, indicated by the solid black line.

The experiment ended after a period of inactivity of the animal of two minutes.

3.3.8. Parallel visual discrimination task

The parallel visual discrimination task was inspired by the paired-associate learning task (PAL). The idea of the PAL-task is defined by a dynamic target, depending on the context. This means for example that stimulus A is rewarded, when it is shown next to stimulus B, but not if it is presented next to stimulus C. In a hypothetic stimulus set, consisting of stimulus A, B, and C, there would be the following reward scheme, shown in Table 4.

Table 4: Reward schemes of a paired-associated learning task and a parallel visual discrimination task

a) Reward scheme in a paired-associated Learning task

b) Reward scheme in a parallel visual discrimination task

a

	Target	Distractor
1	A	B
2	B	C
3	C	A

b

	Target	Distractor
1	A	B
2	C	D

The parallel visual discrimination task had the goal, that the animal was learning that there are two different rewarded targets. They are shown next to their respective distractors. In opposite to the PAL-task, not one stimulus set after the other was learned. From the first session after the pretraining phase, the mice were confronted with two different targets. In this case, the rewarded target was a horizontally orientated sine-wave grating and a vertically orientated grating was the distractor or a 45° orientated grating as a target and a 135° orientated grating as the distractor, respectively. This approach was used to test if there is competitive learning between a stimulus, which is according to neuronal data (Roth et al. 2012) heavily perceived on the level of the primary visual cortex and a stimulus which is less well perceived.

3.3.9. Analysis

The data for every single session was saved in an individual csv-file. They were read in by a python script and individually analyzed. For the parallel visual discrimination task, the mean fraction of correct trials was calculated over animals per session. Differences between the learning curves for cardinal and oblique targets were tested on significance with the Mann-Whitney-U-Test. For the staircase procedure, the orientation discrimination threshold for every session was calculated from the csv-file. To calculate the correlation between the number of trials and the performance, Pearsons correlation was calculated. The difference in the delays in terms of the paired visual discrimination learning was tested for significance with the Wilcoxon test. Orientation discrimination thresholds, as well as the mean delay to react to the stimulus, was compared with the Mann-Whitney-U test.

3.4. Results

3.4.1. Parallel visual discrimination learning

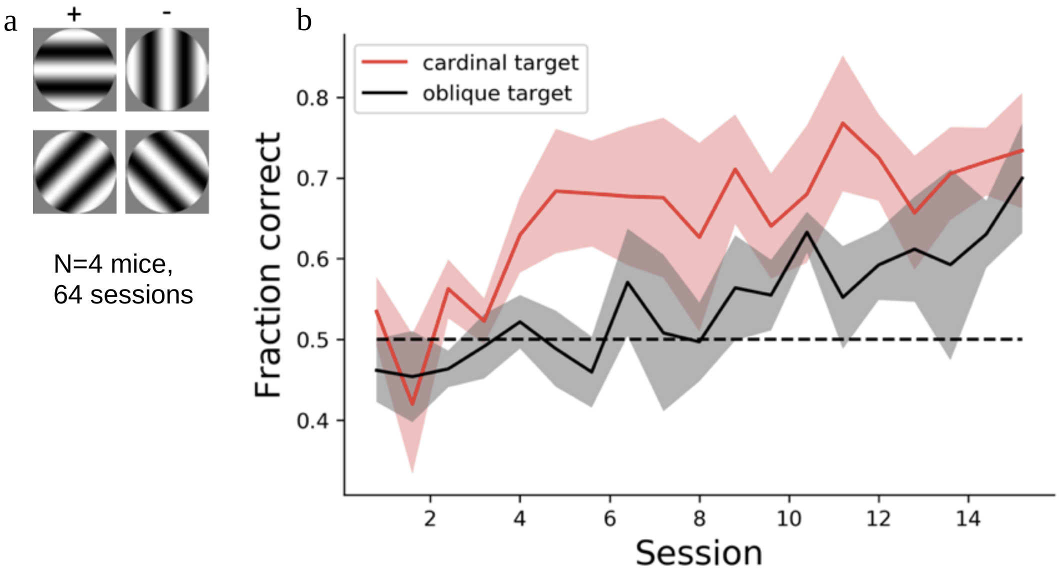


Figure 3.4: Comparison of the performance in the parallel visual discrimination task.

a) Reward scheme in this task. The targets are a horizontal orientated sine-wave grating, as well as a 45° clockwise turned grating. The distractors have an constant orientation with a difference of 90°

b) The black lie indicates the mean performance for the learning of an oblique target, while the red line shows the mean fraction of correct trials for a cardinal target. The shaded areas show the SEM.

In the parallel visual discrimination task (Figure 3.4), the animals showed an initial performance around the guess rate of 50 % of correct responses. A first increase in the performance for the cardinal target (red line) can be observed after four sessions. After the increase, the performance for a cardinal target stays above the performance for the oblique target. (Mann-Whitney-U-Test; statistic=60.0; p=0.0002). They started to equalize after 15 sessions. On average the animals performed in 73,775 ± 2,51 trials per session.

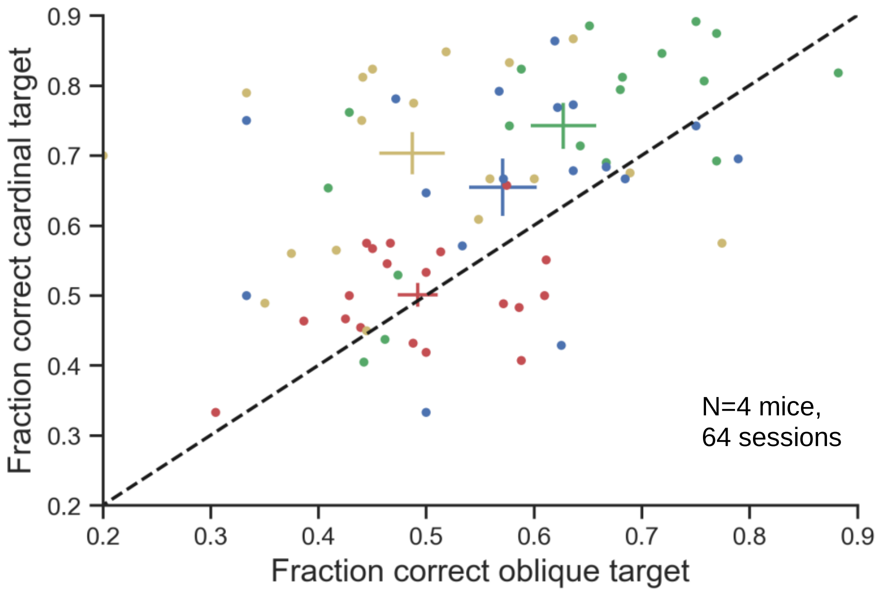


Figure 3.5: Sessionwise comparison for the parallel visual discrimination task. *Every dot represents one session. The colors indicate different mice. The crosses are the average ± SEM performance for a cardinal and an oblique target for every mouse. The used data includes all sessions.*

Figure 3.5 shows the session-wise comparison of the performance for the cardinal and the oblique target, in which every color represents one of the four mice. The crosses indicate the mean ± SEM. Three mice showed a strong bias towards the cardinal target. One mouse, shown in yellow, showed on average a performance of 70 % for a cardinal target, but 50% for the oblique target. The mouse represented by the blue color showed an average performance of 65% for the cardinal target and 58% correct for the oblique target. The highest performance was shown by the mouse shown in green, which reached a performance of 72% for the cardinal target and 63% for an oblique target. One mouse remained at the guess rate for both targets. All sessions above the dashed line indicate better performance for the cardinal target within one session. Dots, which are close to the dashed line indicates sessions without a difference between the cardinal and oblique target.

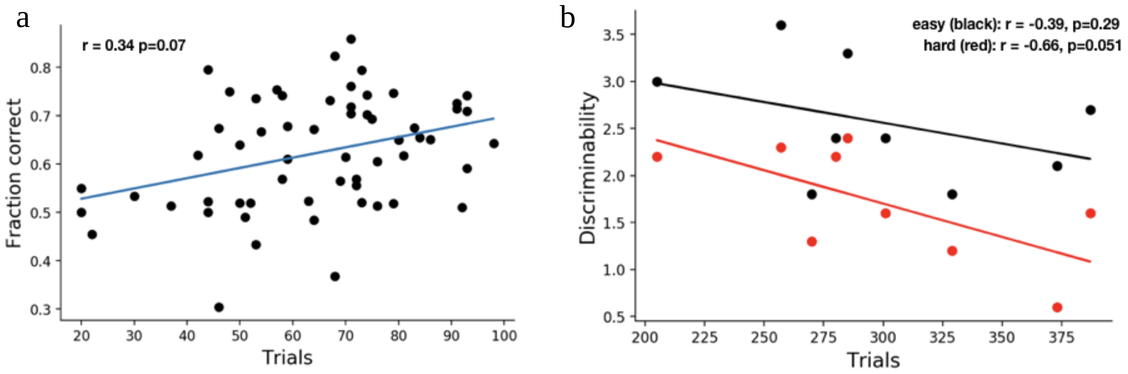


Figure 3.6: Comparison of the performance over trials between the present study and data by Anderman et al. 2010 to show different correlations between the number of trials and the performance/discriminability

a) Data from an orientation discrimination task, presented in this thesis. The fraction of correct trials is shown over the number of trials. Every black dot represents one session. Sessions with more than 100 trials were excluded, since many mice showed a decay in motivation in late trials.

b) Discriminability index over trials in different mice from the studies of Anderman et al. 2010. Black dots represent a session with an easy discrimination task, while the red dots represent sessions with a hard discrimination task

To answer the question if the number of trials has a significant influence on the performance. The data, presented in this thesis (Figure 3.6a) showed a positive correlation between trials and performance (Pearson correlation coefficient; r=0.34, p=0.07). In contrast to that, the data from the literature (Figure 3.6b, adapted from Andermann et al. 2010) did not show a significant correlation between the discriminability and the number of trials. It has to be taken into account, that the number of trials is higher in the head-restrained task by Andermann et al. 2010. There was easy discrimination, with a difference of 90° and hard discrimination, with a difference of 11°, compared. Both showed no significant correlation (Pearson correlation coefficient; easy: r=-0.39, p=0.29; hard: r=-0.66, p=0.051), but there was the trend that there was rather a negative correlation. Due to the respective task designs (2AFC vs GO/NoGo-task), there had to be a comparison between the discriminability and the fraction correct, but this was sufficient to compare qualitative trends.

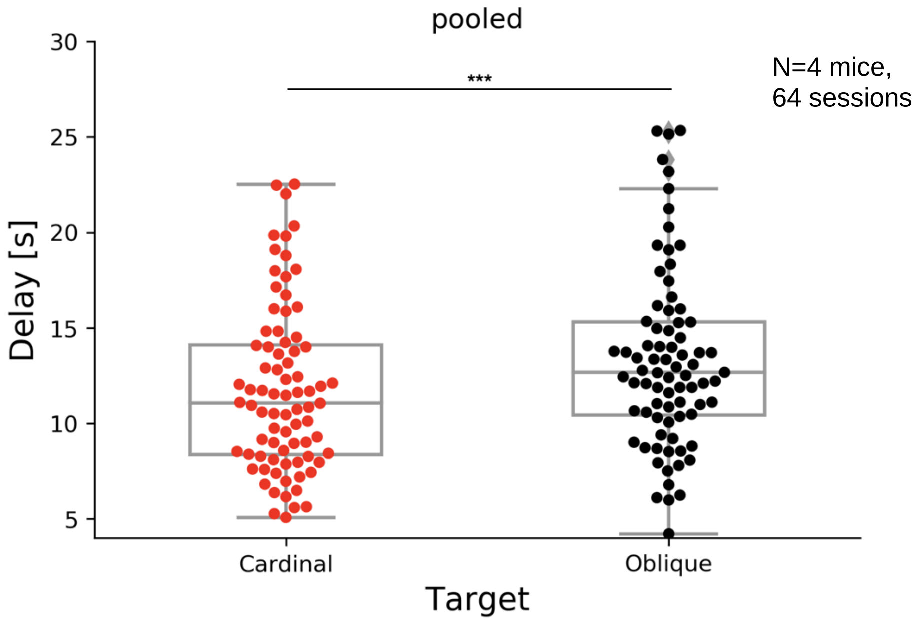


Figure 3.7: Pooled mean delay times to screen touch in the parallel visual discrimination learning over four mice. *All sessions and trials are included. The red dots indicate individual average response delays for each session, representing trials with a cardinal target. Black dots show the individual average response delay for each session including trials with an oblique orientated target. To visualize the distributions the respective box-whisker plots are shown in grey.*

In Figure 3.7 the pooled delay times are shown. This is the delay, the animal needed to touch the screen after the initiation of a trial. There was a mean delay for the cardinal target of 11.78 ± 0.48 seconds. The delay for an oblique target was 13.53 ± 0.57 seconds. The delay time to touch the screen was significantly different between the setting with a cardinal and an oblique target (Wilcoxon=760.0; p=3.71 * 10^{-5}). The animals needed on average a longer time to react to the oblique target and showed a larger range from 4.2 to 32.88 seconds, compared to a range of 5.07 to 22.52 seconds for the cardinal target.

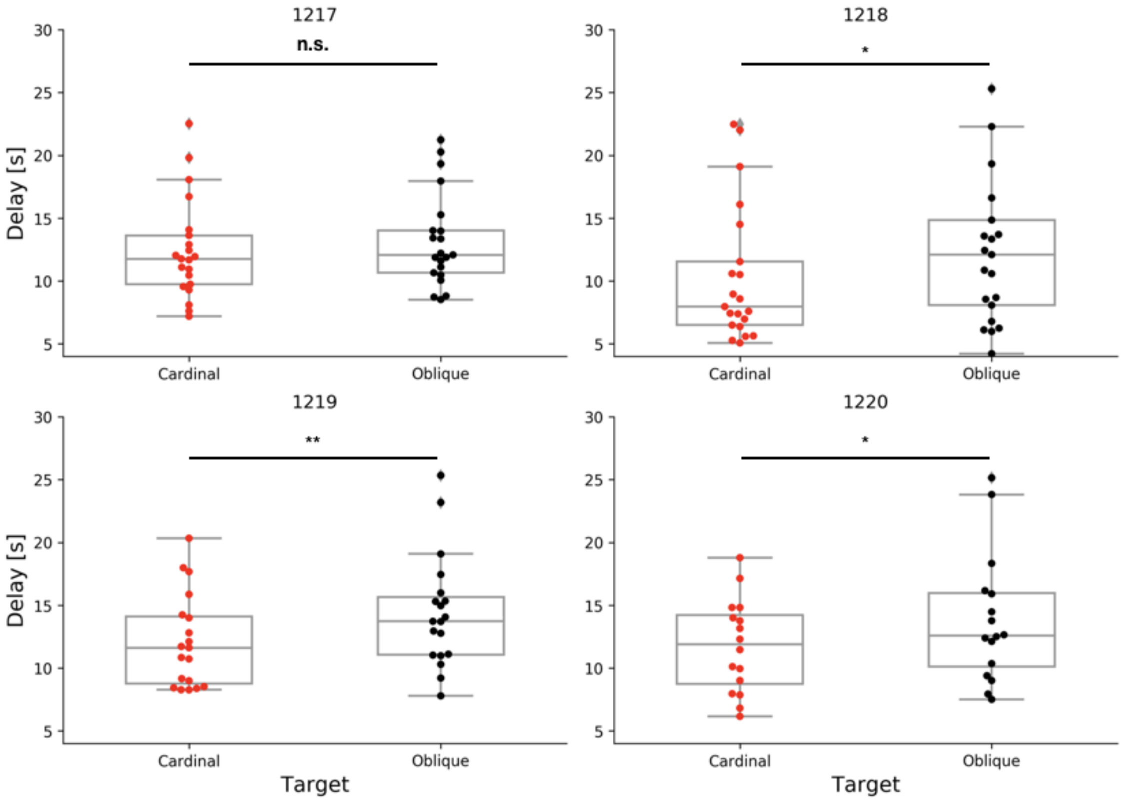


Figure 3.8: Individual delay times to touch a stimulus in the parallel visual discrimination task over four different mice *All sessions and trials are included. The red dots indicate individual average response delays for each session, representing trials with a cardinal target. Black dots show the individual average response delay for each session including trials with an oblique orientated target. To visualize the distributions the respective distributions box-whisker plots are shown in grey.*

The individual comparison showed in Figure 3.8, revealed that the difference between the cardinal and oblique target is not significant in all mice. Mouse 1217 showed a slightly not significant difference (Wilcoxon=63.0, p=0.06), while mouse 1218 showed a significant difference (Wilcoxon=56.0, p=0.03). The strongest difference occurred in mouse 1219 (Wilcoxon=29.0; p=0.008). Even if mouse 1220 did not show any learning progress and no preference for a cardinally or obliquely orientated target, there was a difference in delay times present. (Wilcoxon=20.0, p=0.013). Over three out of four mice, there was a significant trend of longer delay intervals for obliquely orientated targets.

3.4.2. Orientation discrimination with and without retraining

In the next experiment, we wanted to test if the training has a long-lasting effect on the following discrimination tasks. In the initial experiment, we trained a batch of mice (n=6) on a horizontal target. After the animals learned to discriminate between a horizontal and a vertical sine-wave grating, we introduced a weighted staircase procedure. The discrimination thresholds for the horizontal targets are shown in Figure 3.9 and Figure 3.10. In Figure 3.10, the animals (n=4 mice), which were retrained on an oblique target are shown in grey. They were trained on an oblique target of 45° versus the vertically orientated distractor. The vertical stimulus was the distractor in the paradigm they learned before. The idea was, to flip the reward scheme and train the animals on a comparable performance, as they showed in the first orientation discrimination training.

After the animals achieved this performance level, the weighted staircase was introduced again, but with an oblique target. The orientation discrimination thresholds were much higher in this case and were distributed around a threshold close to 35° compared to 20° for the horizontal target (Figure 3.10,U-test=1106.0; $p=1.56*10^{-19}$). The average delay times for each session showed a significant difference as well (Figure 3.12, U-Test=3854.0; p=0.015). In opposite to that, if the training on the horizontal target was skipped and the animals (n=2) were trained on an oblique target from the beginning, they reached the same orientation discrimination thresholds as for the horizontal targets (Figure 3.9, U-Test=2333.0; p=0.31). The response latency did not show a difference outside the retraining-paradigm (Figure 3.11, U-Test=2328.0; p=0.30). This leads to the assumption that the animals attend to the task as before and had no significant decrease in motivation

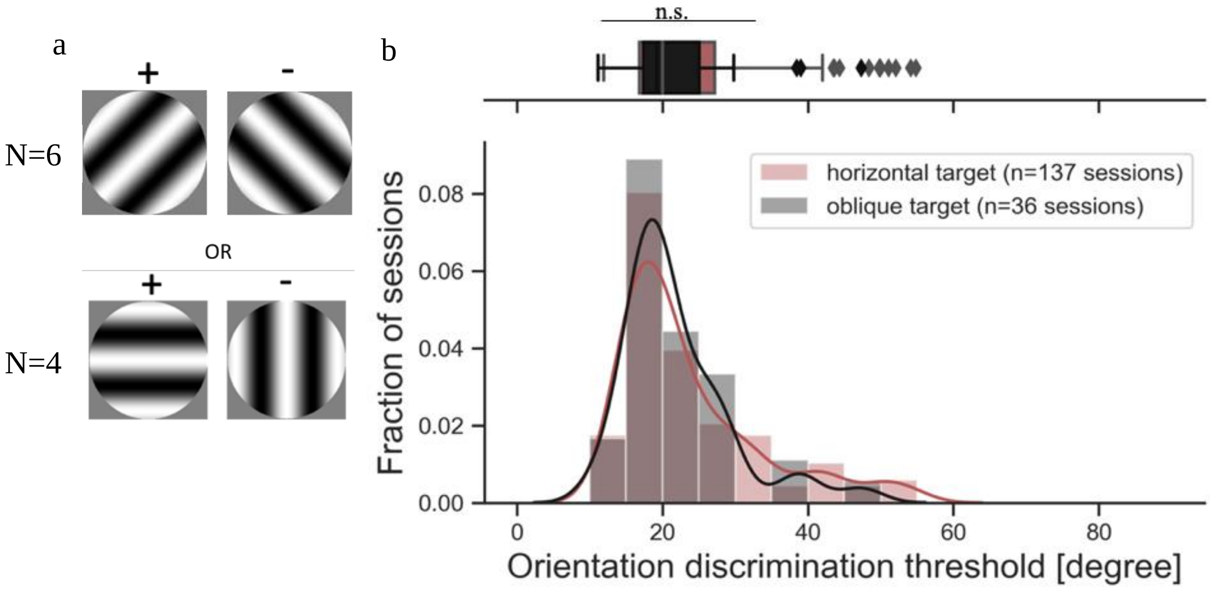


Figure 3.9: Histogram of orientation discrimination thresholds without retraining

a) Reward scheme in this task. There was either a horizontal versus a vertical stimulusset presented or an obliquely orientated target with a 90° clockwise turned distractor during the whole training and testing period. N indicates the number of mice in this kohort

b) Each session is analyzed for the specific orientation discrimination threshold. All orientation discrimination thresholds are shown as a histogram. For better visualization of the distribution, a boxplot is added on top, which represents the same set of data. The red bars and red line indicate the orientation discrimination thresholds for sessions with a horizontally orientated target. The black bars and the black line shows the orientation discrimination thresholds for an oblique target for mice which were trained on an oblique target from the beginning of their task history. There is no significant difference between animals trained on a horizontal target and animals, which were trained on an oblique target.

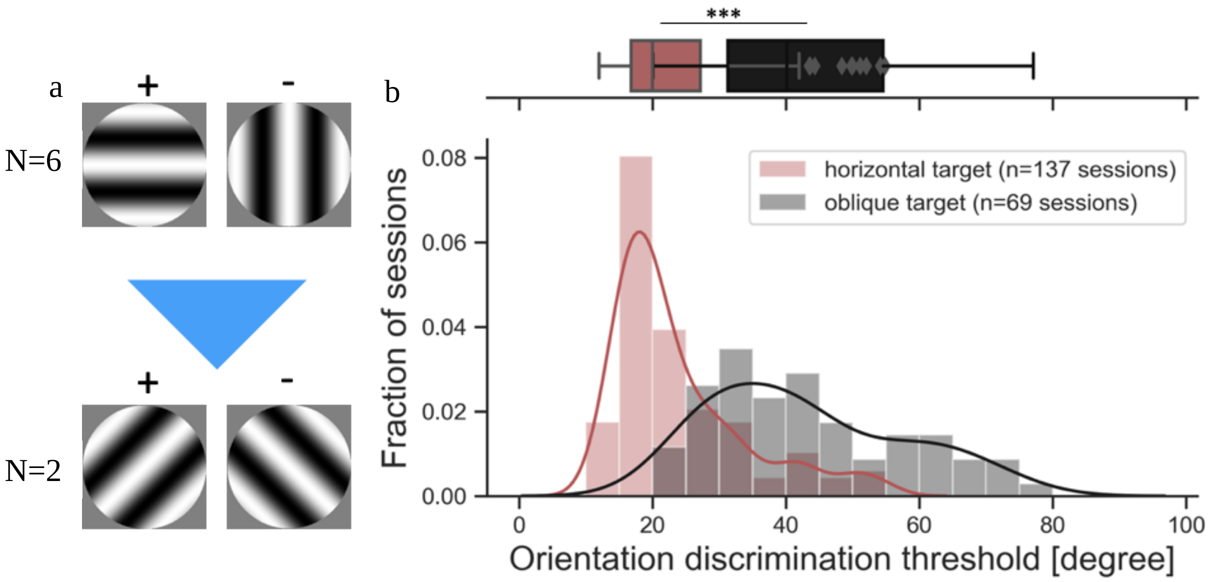


Figure 3.10: Histogram of orientation discrimination thresholds with retraining

a) *Reward scheme in this task. The initial training was done with a set of horizontal and vertically orientated sine-wave gratings. After a retraining, the staircase procedure was performed with two obliquely orientated sine-wave gratings. N indicated the number of mice in this kohort.*

b) *Each session is analyzed for the specific orientation discrimination threshold. All orientation discrimination thresholds are shown as a histogram. For better visualization of the distribution, a boxplot is added on top, which represents the same set of data. The red bars and red line indicate the orientation discrimination thresholds for sessions with a horizontally orientated target. The black bars and the black line shows the orientation discrimination thresholds for an oblique target for mice which were trained on a horizontal target from before, but have been retrained on an oblique target. There is a significant difference between animals trained on a horizontal target and animals, which were retrained on an oblique target after they learned to target horizontally orientated sine-wave gratings*

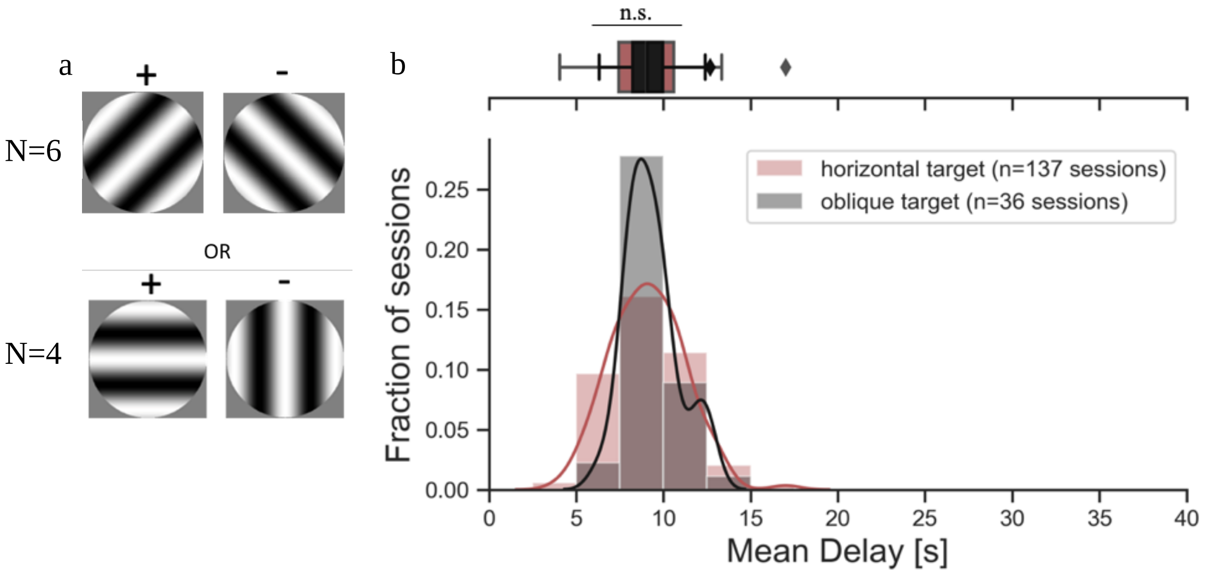


Figure 3.11: Histogram of touch delays without retraining

a) Reward scheme in this task. There was either a horizontal versus a vertical stimulusset presented or an obliquely orientated target with a 90° clockwise turned distractor during the whole training and testing period. N indicates the number of mice in this kohort.

b) The mean delay was calculated for every experimental session and is shown in the histogram. The line plot as well as the box plot on top were added for further visualization of the dataset. The red line and bars indicate the average delay in sessions with a horizontal target, while the black line and bars represent the sessions with an oblique target. There is no significant difference between animals trained on a horizontal target and animals, which were trained on an oblique target.

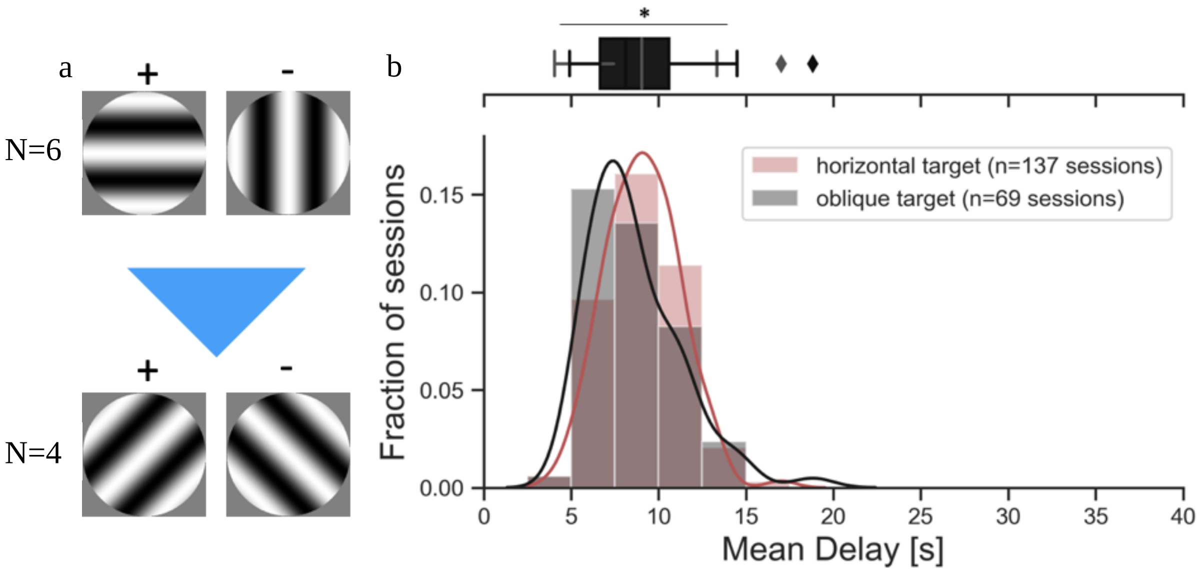


Figure 3.12: Histogram of mean touch delay with retraining

a) *Reward scheme in this task. The initial training was done with a set of horizontal and vertically orientated sine-wave gratings. After a retraining, the staircase procedure was performed with two obliquely orientated sine-wave gratings. N indicates the number of mice in this kohort.*

b) The mean delay was calculated for every experimental session and is shown in the histogram. The line plot as well as the box plot on top were added for further visualization of the dataset. The red line and bars indicate the average delay in sessions with a horizontal target, while the black line and bars represent the sessions with an oblique target. There is a significant difference between animals trained on a horizontal target and animals, which were retrained on an oblique target after they learned to target horizontally orientated sine-wave gratings

In principle, there are two alternatives, which lead to a change in the orientation discrimination threshold. On the one hand, it could be a task history-dependent effect. On the other hand, we hypothesize that there is a change in the tuning of orientation-selective cells in the primary visual cortex. On average the animals performed in the staircase experiment with a horizontal target in 92.76 ± 2.09 trials and in 125.29 ± 2.89 trials with an oblique target.

3.4.3. Summary

- The parallel visual discrimination learning task indicates that cardinal orientations are preferred in terms of learning an orientation discrimination task
- The animals show a higher reaction time to the stimulus for cardinal targets as for oblique targets
- If the animals are trained on the oblique target early from an age of eight weeks, they achieve an equal orientation discrimination performance as for horizontal targets
- If the animals are trained on the cardinal target and get retrained after they learned the task, they are not able to achieve an equal orientation discrimination performance for the oblique target as for the horizontal target
- The reaction time to the stimulus is not affected by the retraining

3.5. Discussion

Orientation tuning in the mouse primary visual cortex in excitatory cells as well as in inhibitory interneurons is a well-known and largely described phenomenon (Lee et al. 2014, Sohya et al. 2007, Roth et al. 2012, Kerlin et al. 2010). On the behavioral level, there is a gap in comparing visual orientation discrimination performance with view on cardinal and oblique orientations. In this chapter, data is presented which shows that mice are better in learning a cardinal target The parallel visual discrimination task gave the evidence, that mice have a higher learning rate when two sets of stimuli with the same difficulty are shown in the task (Figure 3.4).. The learning of a set with two oblique orientated sine-wave gratings was slower. For a further quantification a batch of animals was trained on a horizontal target and the orientation discrimination threshold was measured by a staircase procedure. The same mice performed much worse on an oblique target, after they were retrained (Figure 3.10). To see, if this effect is plastic, the experimental training was altered. Therefore, a new batch of mice were trained on an oblique target from the first training session. In the staircase procedure, these animals showed comparable orientation discrimination thresholds as animals, which were trained on cardinal targets. Therefore, we can conclude that there is a functional role of the neuronal overrepresentation of neurons, tuned for cardinal orientation, but the visual system of mice is able to adapt if other orientations are overemphasized in a task (Figure 3.9).

In a Go-/NoGo task with head-restrained mice (Andermann et al. 2010), it was shown that if the mice are trained on a horizontal target they were able to discriminate reliably at a difference of 11° (d'=1.7). There was no test about the orientation discrimination performance for an oblique orientated target. We were able to show, that our experimental procedure predominantly leads to orientation discrimination thresholds between 15° and 20° over several individuals (Figure 3.9). This proofs our concept to gain a comparable performance in the animals as in a task with head-restrained animals.

Compared to a task in freely behaving mice, the mice performed in more trials, when they are head-restrained (average 299 ± 57 trials). The higher number of trials might also lead to an increase in orientation discrimination sensitivity. Depending on the paradigm the animals performed in 73.775 ± 2.51 trials in the parallel visual discrimination learning task (limited to 100 trials) and in the staircase experiment with 125.29 ± 2.89 trials (oblique target) and 92.76 ± 2.09 trials (horizontal target). According to the study by Andermann et al. 2010, it is not likely that there is a positive correlation between the number of trials and performance (Figure 3.6). In contrast to that, the result of the parallel visual discrimination task showed that the performance is significantly positively correlated with the number of trials. Since the experiment in freely behaving mice, with an overall lower number of trials, showed a different range of trials as the task in head-restrained mice, there is the possibility that the effect decays on a high number of trials and only matters if the animals perform in less than 100 trials per session.

3.5.1. Parallel visual discrimination learning

The general concept of the paired-associate learning task was the idea for a parallel visual discrimination learning task. To our knowledge, in previous visual discrimination experiments, mice got trained on one target instead of two. The difference between the paired-associate learning is the rewarding scheme. Since in the paired-associate learning task, a stimulus that is rewarded in one setting is the distractor in another context (Talpos et al. 2009). In this experiment, two clear sets of targets and distractors are used. This approach gave a first idea of the learning behavior in mice when they have to discriminate between sets of stimuli which also evokes different neuronal activity due to the overrepresentation of cardinal orientations.

It can be shown by the results that the initial learning of visual discrimination relies on the perception of a stimulus (Figure 3.4). A stimulus, which evokes more neuronal activity is learned faster in terms of orientation discrimination. In terms of the sensory processing of orientations, it is likely that the perception reflects the ability to learn the discrimination of a stimulus. Contrast studies have shown, that the performance is increased when the stimuli are well-perceived (Long et al. 2015). Another point is the increased reaction time to the oblique orientated stimulus set (Figure 3.7). It was already shown that there is a correlation between the difficulty of a task and the reaction time (Sanders & Kepecs 2012), while other authors noted the correlation between the reaction time and the skill level of the animal (Humby et al. 2005). According to Sanders & Kepecs, the difference between the easiest and hardest discrimination is at 60 ms (decrease of 21 % for the easier task), while the difference in the present parallel visual discrimination learning task was at 1.8 s (decrease of 12.9 % for the easier task). Indeed, the comparison between head-restrained and freely behaving animal always has a speculating character, but the time difference showed a similar trend in these two experiments. Thus, this is an additional evidence that it was easier for the animals to learn the horizontal target versus a vertical distractor than to distinguish two oblique orientated stimuli with the same orientation difference. It can be also argued, that the animals showed a higher confidence in the trials with a horizontal target, which is also an argument for a difference in the perceptual difficulty of the two stimulus subsets.

3.5.2. Staircase with retraining

After the animals are trained on one target in advance, they showed a significantly worse orientation discrimination if they are retrained on a new target (Figure 3.10), even if the task performance level was comparable on a fixed orientation difference of 45°. The previous findings from the parallel visual discrimination learning task suggest that the animals perceive the orientation less well in oblique target compared to the cardinal orientated targets. This could be a valid reason for the difference in the orientation discrimination task since the neurons in the primary visual cortex tuned for oblique orientations are underrepresented (Roth et al. 2012).

Furthermore, it is possible, that the difference can be explained by the task-history dependent bias of the animal. This means, due to previously learned tasks, the animals would not be able to transfer their learned behavior into a new context. What argues against a task-history dependent bias is the control at a difference of 45°. For this retraining paradigm, they reached similar performance levels for a switched reward scheme. The reversal training paradigm, used by other authors, revealed that animals are able to learn a new reward scheme (Brigman et al. 2009).

We argue for the quantification of a behavioral and functional effect because of a change in the neuronal selectivity. Previous studies were able to show, that neuronal activity can change during a period of training. In one task, mice had to distinguish between a vertical and an angled square-wave grating (Poort et al. 2015). While the vertical grating was rewarded, the number of cells selective for this specific orientation was two-fold increased after an extensive training. A lower, but significant increase was observed for the oblique distractor orientation. This also supported by another study by Goltstein et al. 2013, which showed an increase in tuning bandwidth of orientation selective cells. The sensory input has a huge impact on the cortical representation and as we were able to show, also on the behavioral output of an experiment, when the animals are retrained.

3.5.3. Staircase without retraining

A direct training on an oblique orientated target revealed an opposite effect. After the animals collected their first task experience with the oblique target instead of the initial training on a horizontally orientated target, they showed an equally good performance on orientation discrimination (Figure 3.9). This finding reveals a conflict with the previous results. The paired visual discrimination task, as well as the staircase experiment with retraining, showed an impairment when it comes to the discrimination of obliquely orientated targets. This contradicts the historical findings, that rats show an impairment for the learning of oblique orientations (Lashley 1938, Dodwell 1970), while the parallel visual discrimination task supports these findings. One possibility is, that animals are able to learn orientation discrimination at oblique orientations equally good, but need a longer training period. Due to the high variability in the learning curves, our data do not allow any conclusion with view on the length of the training period. Nevertheless, the parallel visual discrimination task showed a delayed increase in the performance for an oblique target. Another hypothesis is a shift in the neuronal tuning due to the training. This needs to be tested in an experiment using imaging methods, but as mentioned before (Goltstein et al. 2013, Poort et al. 2015) this shift can be induced by training. It remains unclear, how strong an induced shift can be, but judging from the behavioral data, we assume a strong shift towards a stronger selectivity for oblique orientations.

But not just in animals, the oblique effect was described. Also, humans show a decreased discrimination sensitivity for obliquely orientated gratings (Engels & Furmanski 2000). This study linked the results also with a higher evoked activity in the primary visual cortex for cardinal orientations revealed by a functional magnetic resonance imaging approach. The link between neuronal activity and discrimination sensitivity gives evidence for similar processing in the mouse visual cortex. The findings presented in this thesis showed in the first two experiments, that cardinal orientated gratings are easier to learn for mice, which is in line with their orientation tuning in the primary visual cortex.

Thus, comparable to findings in the primary auditory cortex (Xin et al. 2019), where a task-induced tuning shift is present, may suggest similar processes in the visual system.

The reaction times to the stimuli did not show a difference, when the animals are trained directly on an oblique target (Figure 3.11), while there was a difference in the parallel visual discrimination learning task (Figure 3.7). On the first look, this is contradicting each other, because the longer reaction times are not consistent. But the settings are quite different, because in the parallel visual discrimination learning task, a competitive situation is present. In a competitive setting, the learning capacity needs to be allocated and may explain the difference in the reaction time. In the following training on an oblique target, the competitive situation was completely absent, while the animals only had to concentrate on one target.

3.5.4. Conclusion

It can be concluded that there is a functional effect of the neuronal overrepresentation of cardinal orientations. So far, this was not reported in mice until now. This is also in line with the findings of Girshick et al. 2011, who showed that cardinal orientations are overrepresented in natural environments. In two different paradigms, we showed a quantitative and qualitative difference in the learning of cardinal- or oblique-orientated sine-wave gratings as hypothesized from the neuronal findings of others (Roth et al. 2012). In addition, we showed evidence for a neuronal plasticity if an animal is trained on other orientations. This plasticity was already shown via electrophysiological recordings in ferrets (Chapman & Stryker 1993) and cats (Stryker et al. 1978). The calcium imaging study by Roth et al. 2012 presented that cardinal orientations lead to more pronounced responses in the mouse primary visual cortex and posteriomedial area. Together with the analysis of the environment by Girshick et al. 2011, there is already an insight into the adaptation of the mouse visual system to the environment. In the present thesis we quantified this effect and closed the gap for evidence of an oblique effect in mice.

3.6. Outlook

The previous findings give an insight into the perceptual behavior of orientations but also reveals new questions. The most prominent question is a possible shift in the tuning of orientation-tuned neurons in the primary visual cortex. An answer to this question also reveals the limitation of experiments in freely behaving animals. By now, the most convenient way would be an experiment with head-restrained animals to enable imaging while the animal is behaving. The transition of the orientation discrimination task to a task for head-restrained mice is possible. Experiments including imaging approaches in a behavioral task are largely described (Leinweber et al. 2014, Andermann et al. 2010). This experiment would need a tight time schedule because the surgeries has to be planned.

After the recovery, the training of the animal can be started, but it needs more time to habituate the animal to a setup with a head-restriction than the habituation to the touchscreen chamber. It has to be taken into account, that there is the possibility of age-dependent effects. To avoid this delay, it would be worth a try to perform the training of the animals in the touchscreen chamber and transfer them to the head-restricted version of the same task. To our knowledge, there is no data about the transfer between two distinct approaches in a behavioral experiment in this specific setting. The fact, that it is not directly possible to let the animal touch a screen in a head-restrained task, argues against this possibility. Therefore, it can be suggested to redesign the whole task to the head-restrained setting. Comparable to a study in the auditory system (auditory tuning shift) the animals can then be tested for a change in cortical orientation tuning during active and passive behavior. Besides the primary visual cortex, the posterior medial cortex would also be a candidate area to see effects, because it is also known for a strong orientation tuning (Roth et al. 2012).

Chapter 4: Orientation and spatial frequency discrimination in nature-inspired stimuli

4.1. Abstract

The perception of natural stimuli is an ongoing research question. Artificial stimuli like gratings are characterized by a strictly defined characterization of their orientations, spatial frequencies, and contrasts. More enriched stimuli, which add bandwidths to these parameters are used in the research of the visual system as well, but not that prominent in mice. There are ongoing questions, which are described in species with a higher developed visual system like humans and monkeys. One assumed principle is the role of gain normalization in terms of the perception of enriched stimuli. It has been proposed in the human visual system that due to a divisive gain normalization, more information in a stimulus does not necessarily come with a functional advantage. The goal of this chapter is, to test if this is also valid for mice with view on their capability to discriminate between orientations and spatial frequencies in a touchscreen chamber-based 2AFC task.

Motion Clouds are designed to synthesize a well-defined naturalistic stimulus with quantified parameters in terms of orientations and spatial frequencies. They have already been applied in human visual research (Simoncini et al. 2012). Thus, it is possible to test the visual system for parallel processing of an enriched stimulus by activating several sensory channels with this broadband stimulus. Here, we made use of these advantages by applying the Motion Cloud stimulus in our tested visual discrimination paradigm during active behavior. In addition, the neuronal responses were tested by widefield imaging.

Changing the bandwidth of a visual parameter has direct impact on its discrimination since two stimuli would increasingly overlap with increasing parameter bandwidth. Therefore, in one paradigm we tested the spatial frequency discrimination performance with a varying orientation bandwidth. In another paradigm, we tested the effect of different spatial frequency bandwidths on an orientation discrimination task. While we found no clear significant influence of the spatial frequency bandwidth on orientation discrimination, and increased orientation bandwidth led to an improvement on spatial frequency discrimination. For a further investigation, we tested the perception of different spatial frequencies in a passive viewing task measuring the optomotor response. The reflexive response to different drifting stimuli did not depend on their orientation

bandwidths, suggesting a role of the visual cortex in the before found improvement in visual discrimination performance. Therefore, we recorded the neuronal response with wide-field imaging of the entire visual cortex. Responses to presented motion cloud stimuli increased with a larger orientation bandwidth for a fixed spatial frequency of 0.04 cycles per degree, while this effect was not shown for a higher peak spatial frequency of 0.16 cycles per degree. The improved visual discrimination performance might therefore by driven by a stronger recruitment of the visual cortex by naturalistic broad orientation bandwidth stimuli.

4.2. Introduction

4.2.1. Naturalistic stimuli

As described in the general introduction the perception of naturalistic stimuli is a complex problem. Artificial stimuli, like sine-wave gratings are strictly defined. They are synthesized with one specific orientation, spatial frequency and contrast. It is obvious, that this does not reflect the statistics of natural images. To reflect natural images, it needs another level of complexity. To gain principle knowledge on cortical processing of visual stimuli, gabor-patch gratings have a long history, but they do not solve the question after the complex processing of a natural scenery. A natural scenery is an ensemble of orientations, spatial frequencies and contrasts. The field of research of the perception of naturalistic stimuli is facing a principle problem: natural images are normally not well-parameterized. It is possible to analyze naturalistic stimuli with view on different characteristics, but vice versa the synthesis of nature-like stimuli has been a problem. For this approach, so-called Motion Clouds were developed (Leon et al. 2012). Motion Clouds are a framework to synthesize stimuli with predefined settings, so it is possible to determine orientations and spatial frequencies as well as their respective bandwidths. This is a big advantage over the stimulation with natural images containing characteristic structures like a horizon (Yu et al. 2018). In this chapter, we want to introduce the Motion Cloud stimulus to the behavioral paradigms in mice, established in the previous chapters of this thesis. Utilizing the operant conditioning touchscreen chamber presented in this thesis, the orientation discrimination task was used. Besides that, we established a spatial frequency discrimination task. The leading question is, how the bandwidth of one parameter influences the discrimination of another parameter, e.g. the effect of a spatial frequency bandwidth on orientation discrimination. Motion Clouds were already used in other studies (Simoncini et al. 2012) in investigations of the human visual system. A speed discrimination task revealed that an increase of the spatial frequency bandwidth is not linked to a functional advantage. In a series of experiments, we want to test if this is also valid for mice. An extensive two-photon-imaging study revealed strong parallel processing in the mouse visual system (Roth et al. 2012), further described in the next chapter.

4.2.2. Parallel processing

The stimulation with full-contrast square wave gratings with different orientations showed a strong parallel processing in the primary visual cortex as well as in the posteriomedial area of mice (Roth et al. 2012).

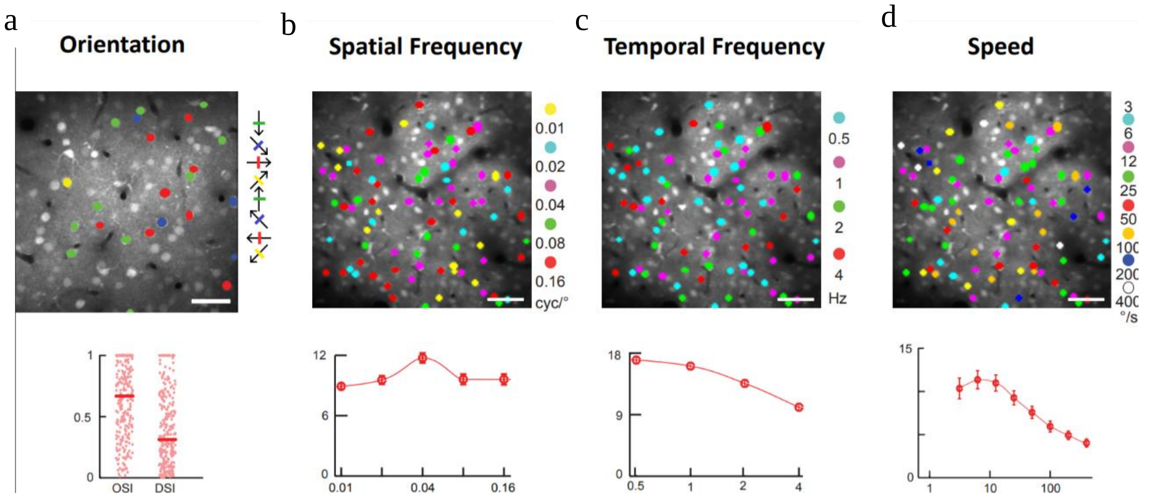


Figure 4.1: Overview of selective neurons in the primary visual cortex (V1)

a) Orientation selective cells are colored in the respective color. Below the distribution of orientation and direction selective cells is shown

b) Spatial frequency selective cells are colored in the respective color. Below the distribution of responsive cells to each tested spatial frequency [cps] is shown

c) Temporal frequency selective cells are colored in the respective color. Below the distribution of responsive cells to each tested temporal frequency (Hz) is shown.

d) Speed selective neurons are colored in the respective color. Below the distribution of responsive cells to each speed [°/s] is shown

It was shown that subsets of neurons react to specific values more pronounced. This applies to different visual inputs, as shown here for orientations, spatial frequencies, temporal frequencies and speed (Figure 4.1) in a two-photon imaging approach. This represented the idea of several sensory channels in the visual system. Similar effects were shown in the same study for different spatial and temporal frequencies. The question arose, how these different sensory channels react to stimuli with enriched bandwidths of orientations and spatial frequencies. Following the findings by Roth et al. 2012 we hypothesize that several channels should be activated if a stimulus contains the respective information. In addition, superposition of gratings with different orientations evoked additional pattern selective responses also in mouse V1 (Muir et al 2015). A study using natural texture stimuli in primates showed that the secondary visual cortex increased its activity during the presentation (Freeman et al. 2013). An analog structure in mice is not described to our knowledge. A further question is the functional effect of the activation of a number of sensory channels. An enriched stimulus provides more information. This could then be reflected in an improved perception and performance in visual discrimination tasks. However, the human visual system did not show a better performance, if more information were provided (Simoncini et al. 2012). Disicrimination of the drifting speed of visual stimuli was not changed with different spatial frequency bandwidths. A possible explanation could be a gain normalization mechanism whereby the increase in gain by additional activated channels is reduced by local inhibition to avoid an overload of information. In this case, the additional information might not lead to a stronger activation or better perception.

4.2.3. Gain modulation

Gain modulation is defined as “a nonlinear way in which neurons combine information from two (or more) sources (…)” (Salinas & Sejnowski 2010). A mechanism of gain modulation is gain normalization, which is suggested as a canonical neural computation (Carandini & Heeger 2012). This principle considers that the responses of neurons have to be reviewed under consideration of the summed activity in a pool of neurons. In the visual system, the first step in this gain modulation is the anatomical environment of the eye. Different amounts of photoreceptors, as well as pupil dilation, modulates the amount of information that passes to the cortical areas for further computation. The photoreceptors increase or decrease their response according to the light intensity in a non-linear way (Baylor & Fuortes 1970, Boynton & Whitten 1970). The general idea in the research of gain modulation and gain normalization is an idea of neural coding, similar to the computation in technical approaches. Thus, the idea of gain modulation also follows ideas from efficient coding (Carandini & Heeger 2012). Avoiding an overload with information by strong stimuli (or bright light in the case of photoreceptors) increases the active range of the sensory system.

The idea is that normalization of sensory inputs results in a higher sensitivity to changes in a dynamic range. One example for this is light sensitivity. The adaptation to light by a modulation of the pupil diameter is a well-described phenomenon (Rodieck 1998).

According to the Weber-Fechner Law, which assumes a constant ratio between the change in the stimulus and the absolute intensity to describe a change in a stimulus, modulative processes can correct for that. Thus, for example, a change of 1 Lux under low light conditions is easier to detect then under high light conditions. The normalization of the sensory input leads to the fact, that the relative change is crucial for the detection of changes.

4.2.4. Discrimination

Different stimuli can evoke different responses. In a discrimination task, the computation is based on the comparison between these neural responses. Assuming that every stimulus-evoked activity is represented by n neurons in an n-dimensional space, a linear classifier can distinguish between the responses to different stimuli by drawing hyperplanes between the clusters (Carandini & Heeger 2012, Ringach 2010, DiCarlo & Cox 2007). This leads to a mathematical problem if one stimulus evokes strong responses and another stimulus evokes weak responses. A classifier will define the border either close to the strong or the weak stimulus. This leads to the fact that the classifier will perform poorly if data far away from the border has to be classified. To prevent this the neural responses should be normalized and give the responses a homogenous distance from the offspring.

4.2.5. Natural image statistics

Natural images follow on average a specific distribution of parameters. In the present thesis, the most important parameters are orientation, spatial frequency and contrast. A range of studies analyzed bigger datasets from image databases to draw general conclusions for natural image statistics. Orientations showed a distribution with an overrepresentation of horizontal and vertical orientations (Mazer et al. 2001, Girshick et al. 2011). The orientations were normally distributed around the cardinal orientations. A normal distribution as well was seen in terms of the contrasts within an image. While very high and very low contrasts are underrepresented, most contrast information was shown in the mean range (Ruderman 1994, Bex & Makous 2002). Spatial frequencies are not normally distributed in natural images. They follow a lognormal distribution, with a high number of low spatial frequencies and a decreasing number of higher spatial frequencies (Field 1987, Ruderman 1994, Mazer et al. 2001, Bex & Makous 2002). To represent this natural image statistics, it is needful to move away from Gabor-patch gratings. A proposed approach for the transition to a more naturalistic stimulation is the so-called Motion Cloud stimulus.

4.2.6. Motion Clouds

Motion Clouds were an approach by Leon et al. 2012 to represent natural image statistics in a parameterized stimulus (Figure 4.2). The basic idea behind a Motion Cloud was, that a sine wave or square wave grating has fixed components (Figure 4.3). Thus, spatial frequency, orientation information, contrast, and speed are fixed components. Natural images or movies show characteristic distributions of these components. Natural images show an orientation distribution, which is normally distributed around the cardinal orientations of 0°, 90°, 180°, and 270°. Orientations in between the cardinal orientations are underrepresented (Girshick et al. 2011). Spatial frequencies show a lognormal distribution in natural images (Dong & Attick 1995). A stimulus that adds different distributions of these components can give a more comprehensive approach for experiments in the visual system. It has also been argued that the visual system is evolutionarily adapted to these natural image statistics (Simoncelli & Olshausen 2001).

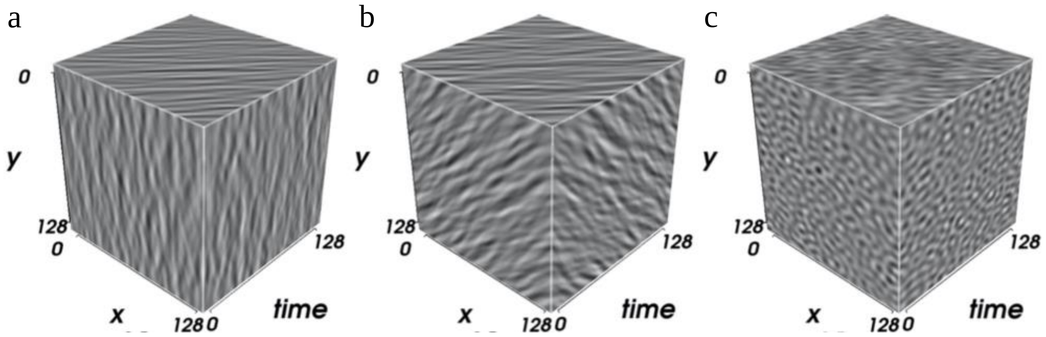


Figure 4.2: Motion Cloud cubes (adapted from Leon et al. 2012)

a) Motion Cloud as a spatiotemporal cube with a vertical orientation over time and movement over time

b) Motion Cloud as a spatiotemporal cube with an oblique orientation of 45° and movement over time

c) Motion Cloud as a spatiotemporal cube with a maximal orientation bandwidth and movement over time

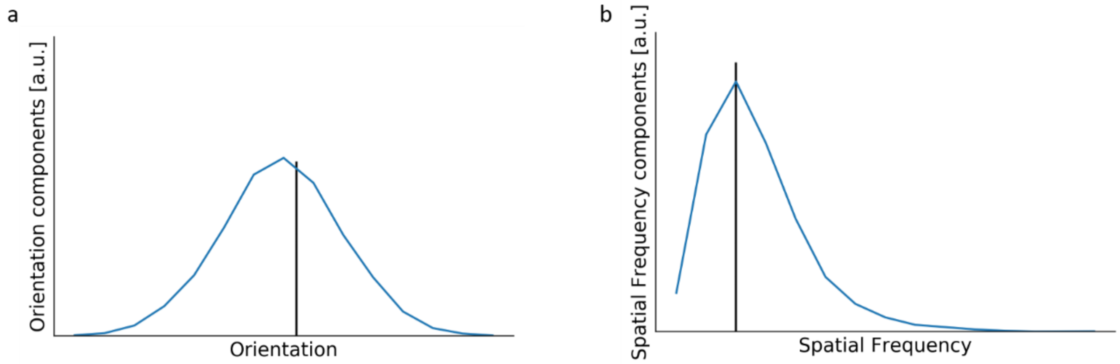


Figure 4.3: Comparison of distributions of orientations and spatial frequencies

a) Orientation components in square-wave gratings (black) and motion clouds (blue)

b) Spatial frequency components in square-wave gratings (black) and motion clouds (blue)

4.2.7. Motion Clouds and Gain normalization

Various studies showed that it can be assumed that the perceptual system is based on a multichannel idea. This means, that individual neurons respond to specific parameters of orientations or spatial frequencies, hence representing different processing channels (Roth et al. 2012). A sinusoidal grating stimulus would, following this idea, activate only specific channels. In opposite to that an enriched stimulus, like the Motion Cloud would activate more processing channels. The ongoing question is about the effect of the activation of various channels on the computational and functional level. Simoncini et al. 2012 proposed that due to the overall inhibitory reduction caused by gain normalization, the activation of a wider range of channels did not give a functional advantage. In a speed discrimination task, they found even an impairment for stimuli with large spatial frequency bandwidths, while the ocular following movement was faster in a short time windows after the stimulus onset. These findings led to the proposal of a model.

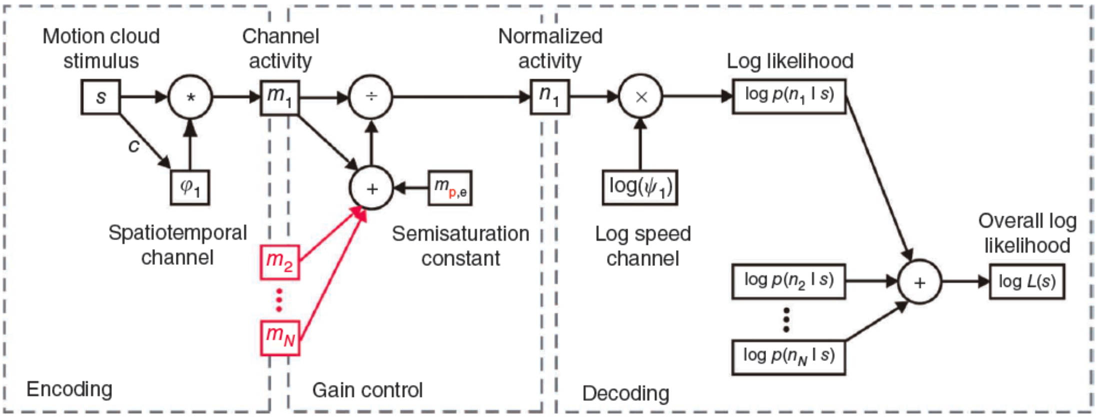


Figure 4.4: Proposed gain normalization model by Simoncini et al. 2012. *The first part on the left shows the encoding of a stimulus by several spatiotemporal channels. This leads to the activity of distinct sensory channels which are computed via summation and a semisaturation constant factor. The gain control happens via divisive normalization. The normalized activity is decoded via a logarithmic likelihood function. The sum of this log-likelihood functions of the distinct sensory channels results in an overall log-likelihood, which represents the perception of the stimulus.*

The model (Figure 4.4) shows how the Motion Cloud is stimulus is encoded and decoded by the visual system with a gain control mechanism. It can be assumed that the motion cloud stimulus activates at first specific spatiotemporal channels according to the stimulus parameters. An encoding modulation happens by the contrast c. The activation of the channels leads to the second stage, where the gain control happens. Every channel is normalized individually by a Naka-Rushton function including the semi-saturation constant m_e and the channel activity corresponding to the contrast c and the bandwidth b (Eq. 8).

$$n_i(c,b) = \frac{m_i^2(c,b)}{m_e^2+m_i^2(c,b)} \qquad (8)$$

According to the task they used, either the ocular following task or the speed discrimination task, two different semisaturation constants are used. After the gain control, the normalized activity is decoded. Therefore, the normalized activity is multiplied with the logarithm of the perceptual channel. In this way, the model provided a log-likelihood for every perceptual channel through the comparison of tuning properties and actual evoked channel activity. Pooled overall activated channels the model derived an overall log-likelihood.

This study covers the question for stimuli with different spatial frequencies and different speed components. It remains unclear, how the orientations can interact in this model since it can be expected that there are similar modulation processes in terms of orientation perception.

4.2.8. Goal

The goal of this chapter was to examine the difference between artificial and nature-inspired stimuli and to investigate the question if there is multichannel processing in the mouse visual system. Therefore, the motion cloud stimulus is utilized to stimulate different channels if they are present. The follow-up question is if there is a behavioral effect if more channels are activated in an active and a passive task. We want to put the model proposed by Simoncini et al. 2012 to a test with mice allowing the combination of perception tests with direct neuronal recordings. To validate the idea of the multichannel idea related to a gain normalization process, a two-alternative forced-choice task was applied. Following the results of Roth et al. 2012, we expected to activate several visual channels with the motion cloud stimuli. If there is a gain normalization process, as proposed by Simoncini et al. 2012, we would not expect to see an improvement in perception or visual discrimination performance. But if there is a behavioral effect, it might be necessary to rethink the gain normalization model for mice. To avoid contamination of the visual discrimination performance by ambiguity of the visual stimuli, we separated the task into two parts. First, the behavioral task contained the discrimination of orientation, similar to the task in Chapter 3, using motion clouds with varying spatial frequency bandwidth. Second was a spatial frequency discrimination task with varying orientation bandwidths of the motion cloud stimuli. To support the behavioral findings from the active task, a passive optomotor response measurement is added. To gain an insight into the neuronal computation of the motion cloud stimulus, a wide-field imaging experiment was conducted.

4.3. Methods

4.3.1. Animals

The orientation discrimination task was performed in six male C57BL/6 mice, starting with the experiments at an age of eight weeks with the pretraining on the discrimination of oblique orientated sine-wave gratings (compare Chapter 2). The experiments were authorized in the licenses 84-02.04.2016.A357 and 84-02.05.40.17.055. The spatial frequency discrimination experiment was performed with six male C57BL/6 mice at the age of 8 weeks at the beginning of the experiments. They were bred and kept at the RWTH Aachen University. During the experiment, the animals were kept on a turned Day/Night-cycle of 12h:12h. The animals are water restricted through the experiment. They receive water *ad libitum* on one day of the week on which no experiments are performed. Per day they receive at least 1.5 ml of water to maintain their wellbeing. They have access to food *ad libitum* all the time. The mice are weighed and observed before the experiments start. The animal is scored for deviant behavior to check for stress symptoms, injuries or other relevant symptoms. This includes the activity of the animal, the movement, the breathing frequency and skin turgor. Minor impairments are scored with one point, major impairments are with two points. If there are no major impairments observed, the animal has to be monitored carefully if the cumulative score is between one and four. At a cumulative score of 5 or higher or a major impairment the animal has to be removed from the experiment and has to be euthanized. At no point of the experiment a euthanasia was conducted. The wide-field imaging experiment was performed in three mice, which had a cranial window above the primary visual cortex and the posteromedial cortex. The surgeries were performed at an age of seven weeks, after two weeks of recovery and handling they were habituated to a head-restrained setup. The wide-field experiment was performed at the age of 16 weeks. The animals were not water-deprived during the experimental phase.

4.3.2. Optomotor response

The optomotor reflex of mice was measured in a setup inspired by Kretschmer et al. 2013 (Figure 4.5a-b). Four monitors were unframed and assembled in a square. In the middle of the four monitors, a platform for the mouse was placed. The floor was a mirror to create the impression of a larger height to avoid the mouse jump off the platform (Figure 4.5c-d). The drifting stimulus was either presented as drifting stimulus via PsychoPy (Peirce 2007, Peirce 2009) or as a video. On top of the box, a camera (Logitech C920) is assembled to create movies of the mouse from above. The videos were analyzed with the Python-package DeepLabCut (Mathis et al. 2018). Therefore, a deep learning algorithm was trained to detect the ears and the tail of the mouse. From this information, it was possible to derive the viewing direction. Therefore, the middle of the platform was considered as the origin in the video. By calculating the function of the line between the ears, the perpendicular line was calculated to indicate the viewing direction. Every stimulus was played for two minutes in a randomized order to avoid adaptation effects.

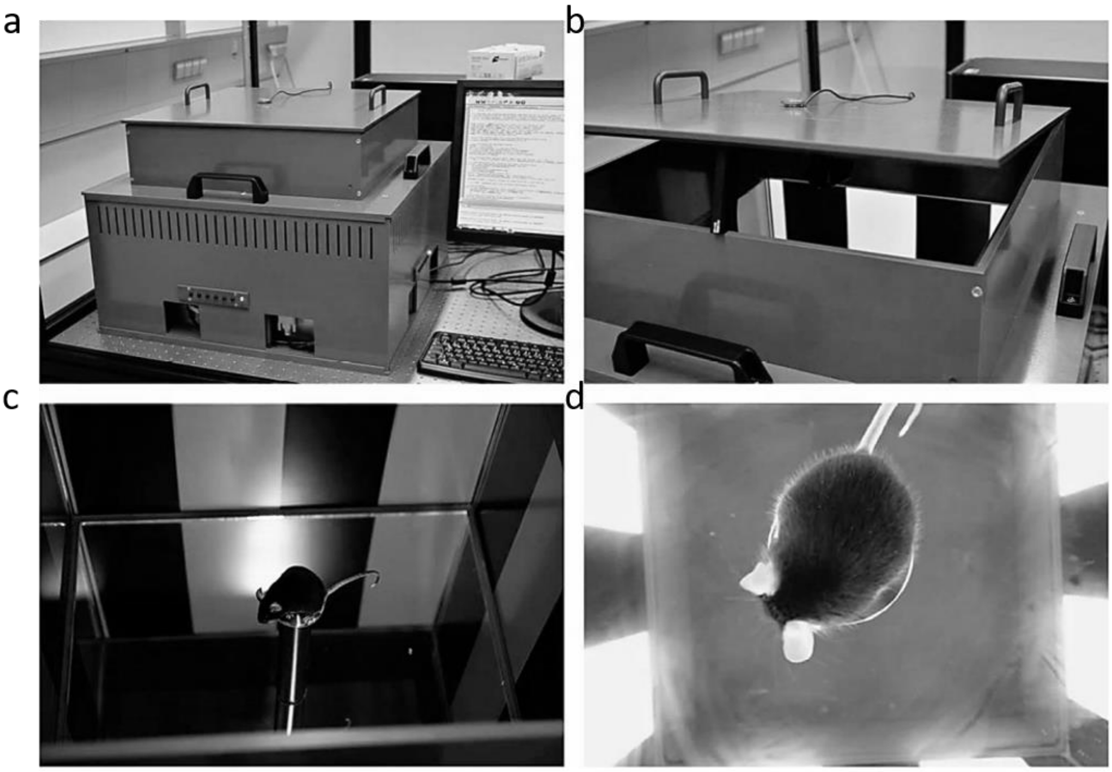


Figure 4.5: Overview of the used optomotor response box

a) Perspective from outside with a closed cap

b) Perspective from outside with an open cap

c) Photograph of a mouse on the platform

d) Top-view, which is used for the analysis

4.3.3. Orientation Discrimination Task

The orientation discrimination task was performed in the touchscreen chamber described in Chapter 2. In the first phase of pretraining, the animals were habituated to the touchscreen chamber. After two consecutive sessions of passing the criterion of one collected reward per minute, the animals proceed to the second stage, where they had to learn to touch the screen in order to get a reward. Again, after they passed the criterion to perform at least one trial per minute on two consecutive days, the training on sine-wave gratings with a spatial frequency of 0.04 cpd with an orientation of 45° or 90° were started. After they gained stable performances on the orientation discrimination, the staircase procedure was started with three different steady Motion Clouds of different spatial frequency bandwidths within the weighted staircase procedure. There was one spatial frequency bandwidth per experimental session used. Following the results from chapter 2, a difference between mice that were trained at 90° or 45°, respectively, was not expected.

4.3.4. Spatial Frequency Discrimination Task

The spatial frequency discrimination task was performed in the touchscreen chamber described in Chapter 2. In the first phase of pretraining, the animals were habituated to the Touchscreen Chamber. After two consecutive sessions of passing the criterion of one collected reward per minute, the animals proceed to the second stage, where they had to learn to touch the screen in order to get a reward. Again, after they gained the criterion to perform at least one trial per minute on two consecutive days, the training on MotionCloud stimuli with a central spatial frequency of 0.04 cpd was started. Next to the rewarded stimulus, a distractor with another spatial frequency was shown. First, it was measured at which spatial frequency discrimination is possible for the mouse. Once the threshold (0.16 cpd for the distractor) was found, drifting MotionClouds (1 Hz) with different orientation bandwidths were introduced.

4.3.5. Stimuli

Motion Clouds are developed to follow the approach to create parameterized natural stimuli. Therefore, a Fourier transformation is created in a three-dimensional space (Eq. 9).

$$I(x, y, t) = F^{-1}\{\varepsilon\beta(f_x, f_y, f_t) * e^{i\Phi(f_x, f_y, f_t)}\} \qquad (9)$$

I corresponds to the luminescence of every pixel with the coordinated x and y at time t. εβ contains the predefined image statistics for every image.

$$\varepsilon\beta = \mathrm{V}(V_x, V_y, B_v) \times G(f_0, B_f) \times O(\theta, B_0) \times C_{(\propto)} \qquad (10)$$

The function V sets the parameters for the motion over time for the stimulus (Eq. 10). It contains the speed in x- and y-direction as well as a speed bandwidth B_V. G determines the spatial frequency. F_0 is equal to the dominant spatial frequency, while B_f adds a lognormal-distributed bandwidth. O is equal to the orientation properties. Therefore, a central orientation is given by θ and adds a normal distributed bandwidth B_0. The realization of the motion clouds was made by the MotionClouds package for python (Perrinet 2014).

4.3.6. Analysis

The datasets from the behavioral experiment are stored in individual csv-files for each session and mouse. They are read out via a specifically written python-script. The videos from the optomotor experiment are recorded with Open Broadcaster Software Studio and are analyzed with DeepLabCut. The readout of DeepLabCut is analyzed with python scripts to derive the shown data by a frame-by-frame comparison. The velocity of head movement is statistically analyzed with a two-way ANOVA and the least significant difference test (LSD). A two-way ANOVA was used as well to analyze the statistical effect of the spatial frequency on the number of head movements. Wide-field data is analyzed with a Mann-Whitney-U-Test. The performance values from the spatial frequency and the orientation discrimination task were tested with a Kruskal Wallis test to see a general effect of the bandwidths, but the conspecific groups were tested individually against each other with a Mann-Whitney-U-Test. The difference between the orientation discrimination in sine-wave gratings and motion clouds were tested with a Mann-Whitney-U-Test.

4.4. Results

4.4.1. Image statistics

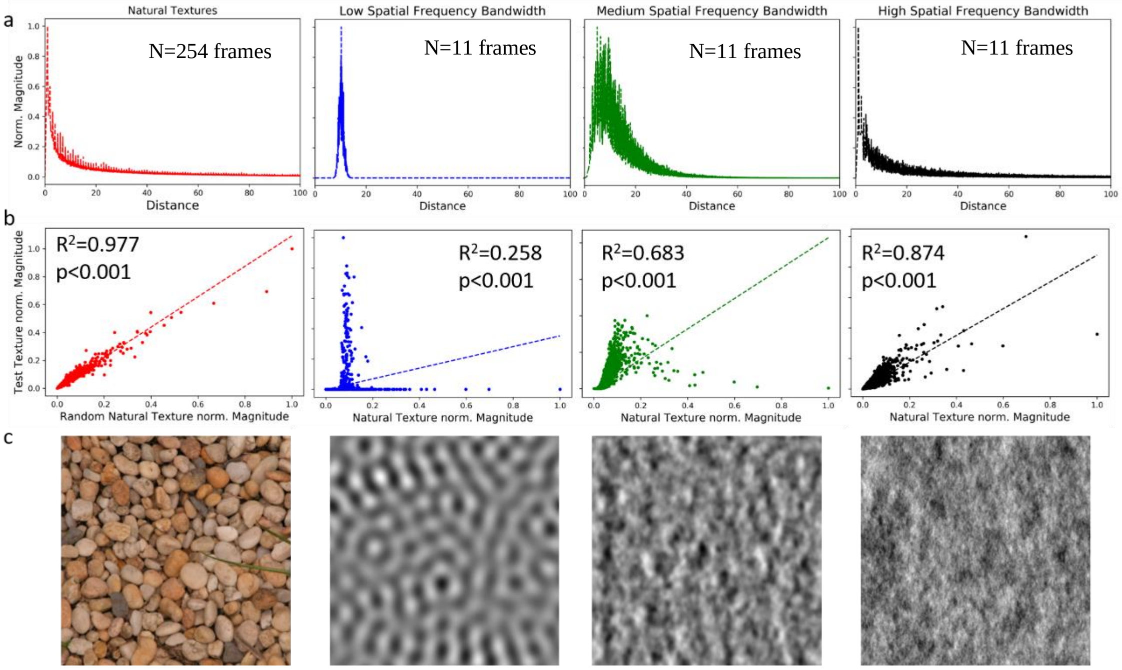


Figure 4.6: Spatial frequency distribution in natural textures and motion clouds.

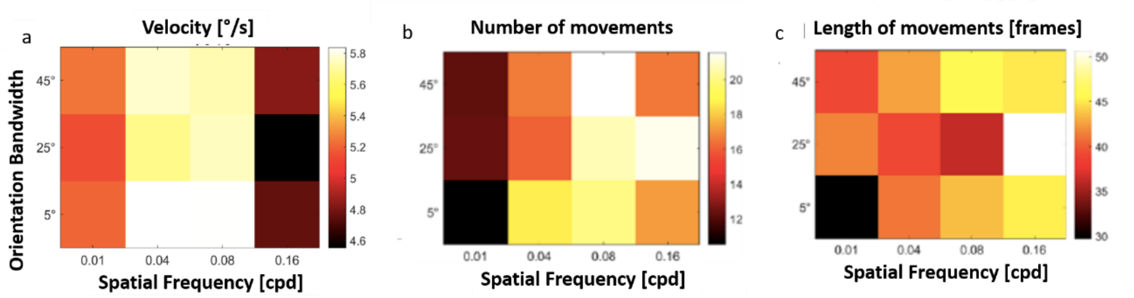
a) Distribution of spatial frequency. This was made by a Fourier transformation and the following calculation of the euclidean distance of single frames from each image subset.

b) Linear correlation between the indicated spatial frequency distribution and the spatial frequency distribution of natural texture.

c) Example stimuli from the image dataset

The spatial frequency distribution in natural textures showed a lognormal distribution with a high bandwidth in spatial frequency. The test of how comparable natural textures were to each other was made by dividing the images from the image database into two halves and comparing them. They showed a highly significant linear correlation, which showed that randomly chosen natural texture images show equal distributions in the spatial frequency. The motion cloud with a low spatial frequency bandwidth showed a distribution which differed from naturalistic images. There was a sharp peak for the central spatial frequency. Around the central spatial frequency, there were no detected spatial frequencies. Thus, there was no strong correlation between the spatial frequency distribution of natural textures and motion clouds with a low spatial frequency bandwidth. Nevertheless, the correlation was weak but significant. For a medium spatial frequency bandwidth, the central spatial frequency was still clearly visible but showed a broader distribution and a characteristic lognormal distribution. There was a stronger correlation between the spatial frequency distribution in natural textures and motion clouds with a medium spatial frequency bandwidth, especially for lower spatial frequencies. Motion Clouds with a high spatial frequency bandwidth, which was higher than the central spatial frequency, showed a shift in the central spatial frequency to lower values due to the arithmetic moment in lognormal distributions with high bandwidth. This stimulus showed a high linear correlation to the natural texture images.

4.4.2. Optomotor response



N=6 mice

Figure 4.7: Optomotor response according to different spatial frequencies and orientation bandwidths.

a) Velocity of following movements over Spatial frequencies and orientation bandwidths.

b) Number of following movements over spatial frequencies and orientation bandwidths.

c) Length of movements in frames over spatial frequencies and orientation bandwidths

The velocity in the following movement showed a higher speed for the spatial frequencies of 0.04 cpd and 0.08 cpd than for spatial frequencies of 0.01 cpd and 0.16 cpd (Two-Way ANOVA: p<0.001). On average, the mice moved their head with a velocity of 5.6 °/s for 0.04 cpd and 0.08 cpd. Along with the orientation bandwidth, no significant influence on the velocity was found. The following movements for the highest spatial frequency of 0.16 cpd were lower than the velocity for the lowest spatial frequency of 0.01 cpd (Figure 4.7a,Least significant difference (LSD test): p<0.01). The number of movements showed also that at the spatial frequencies of 0.04 cpd and 0.08 cpd the most following movements did occur. The number of following movements was significantly different over spatial frequencies (Figure 4.7b,Two-Way ANOVA: $p<0.001$). The longest movements were shown for a spatial frequency of 0.16 cpd and an orientation bandwidth of 25°, while the shortest following movements were shown for a spatial frequency of 0.01 cpd and an orientation bandwidth of 5°. There was no visible trend that the length of the following movement differed over spatial frequency or orientation bandwidth (Figure 4.7c, Two-Way ANOVA: $p<0.23$).

4.4.3. Wide-field imaging

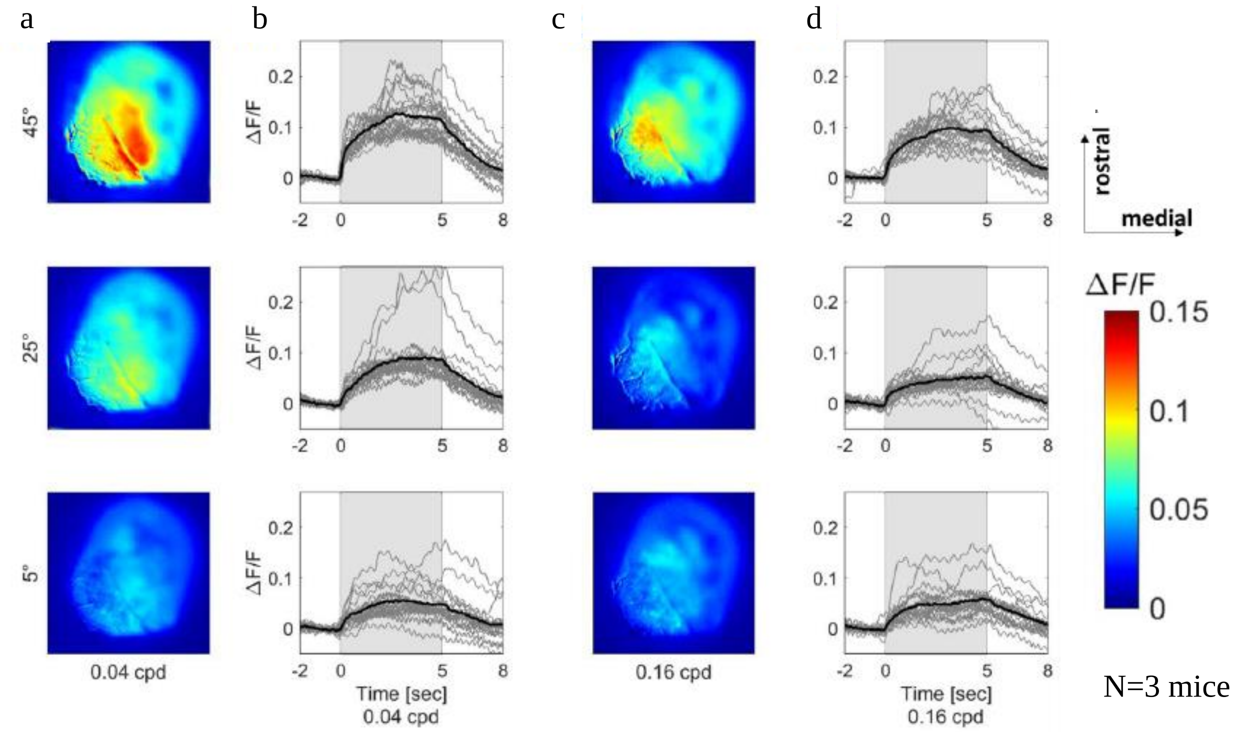


Figure 4.8: Results of the wide-field imaging under the stimulation with motion clouds with two spatial frequencies at different orientation bandwidths.

a) *The raw images from wide-field imaging are shown for a center spatial frequency of 0.04 cpd*

b) Quantitative activity depending on the stimulus presentations for orientation bandwidths of 45°, 25° and 5° for a spatial frequency of 0.04 cpd.

c) The raw images from wide-field imaging are shown for a center spatial frequency of 0.16 cpd

d) Quantitative activity depending on the stimulus presentations for orientation bandwidths of 45°, 25° and 5° for a spatial frequency of 0.16 cpd

The wide-field imaging (Figure 4.8a-b) showed an increase in activity at 0.04 cpd with an increasing orientation bandwidth. The behavioral results already suggested good visibility of this spatial frequency. The average activity for a Motion Cloud stimulus with a spatial frequency of 0.04 cpd and an orientation bandwidth was on average at a ΔF/F of 0.04 %. For an orientation bandwidth of 25°, the increase was a ΔF/F of 0.06 %, while the highest orientation bandwidth of 45° evoked the highest change in activity by 0.09%. For a MotionCloud stimulus at a spatial frequency of 0.16 cpd, the overall activity was similar or lower than for a spatial frequency of 0.04 cpd. The activity for the stimulus with the highest orientation bandwidth was again the highest for this spatial frequency but showed a weaker evoked activity than for the stimulus with a spatial frequency of 0.04 cpd. For the spatial frequency of 0.16 cpd the activity evoked activity of an orientation bandwidth of 5° and 25° did not show a significant difference, while the activity was significantly higher for an orientation bandwidth of 45° (Figure 4.9).

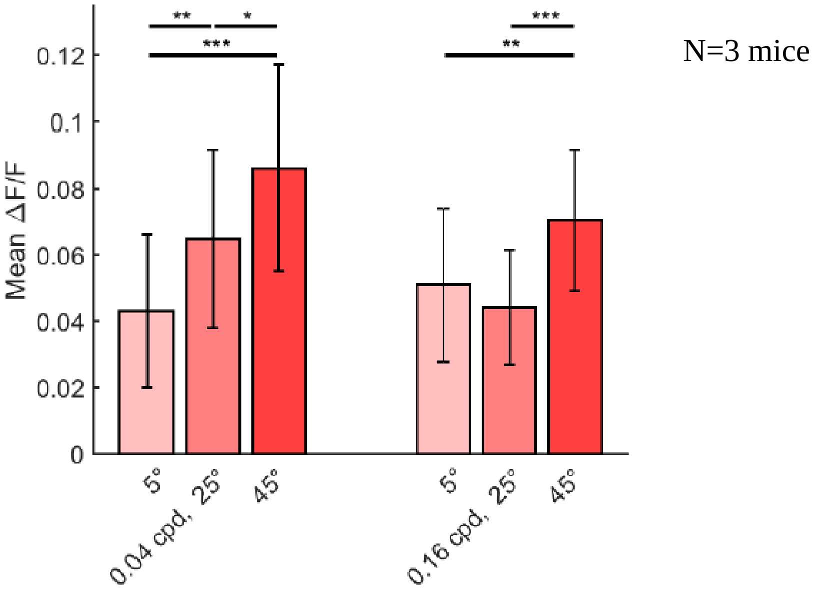


Figure 4.9: Mean change in Ca^{2+}-fluorescence over different orientation bandwidths tested at a spatial frequency of 0.04 cpd (left) and 0.16 cpd (right). *The bars indicate the mean ± standard deviation change in activity when the stimulus is presented compared to the baseline. A constant increase linked to the orientation bandwidth is shown for a spatial frequency of 0.04 cpd.*

4.4.4. Spatial Frequency Discrimination

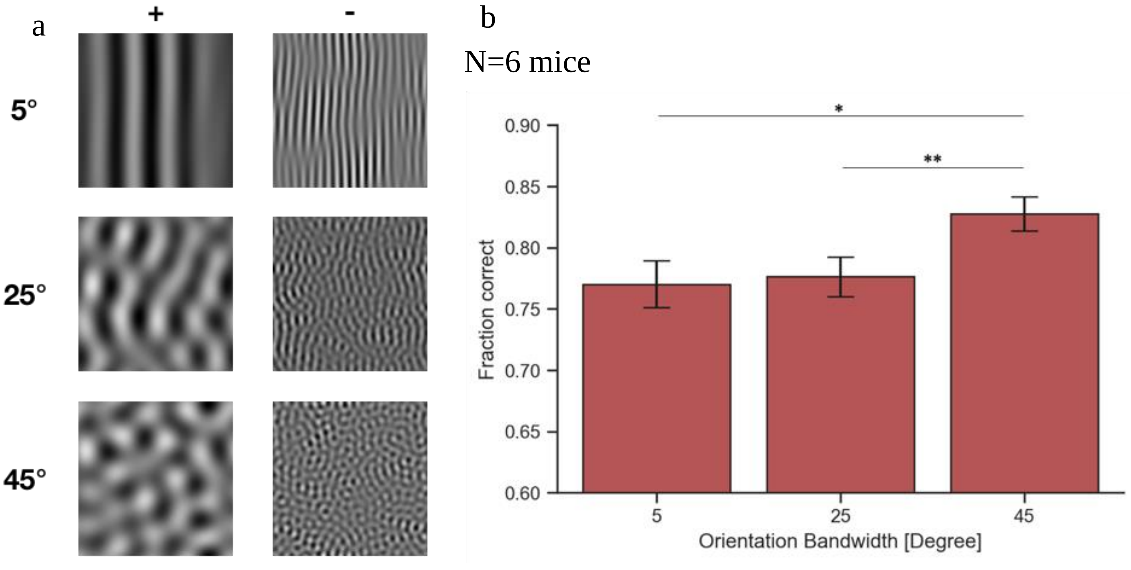


Figure 4.10: Fraction of correct trials in the spatial frequency discrimination task for different orientation bandwidths.

a) Reward scheme of this experiment with example frames. The rewarded stimuli are marked with + and the disctractors are marked with -. On the left side the orientation bandwidth is shown

b)The fraction of correct trials was recorded to see perceptual differences between the orientation bandwidths and their influence on the spatial frequency discrimination capability of mice. There is a significant increase in the performance for the highest used orientation bandwidth of 45°

In the behavioral experiment the performance for the discrimination between a target motion cloud with a spatial frequency of 0.04 cpd and a distractor with a spatial frequency of 0.16 cpd was tested (Figure 4.10b). The orientation bandwidth was randomly chosen trial by trial out of orientation bandwidths of 5°, 25° and 45° (Figure 4.10a). The average performance levels (mean ± SEM) out of 46 sessions in six mice are shown. The performance for the discrimination between two distinct spatial frequencies was significantly higher for a high orientation bandwidth (Kruskal Wallis test=7.566; p=0.023). The highest orientation bandwidth of 45° showed also in specific differences to both other groups (45° vs 5°: U-Test=780.0; p=0.015; 45° vs 25°: Kruskal Wallis test=728.0; p=0.005) while there was no difference between the orientation bandwidths 5° and 25° (U-Test=1056.5; p=0.49).

4.4.5. Orientation discrimination

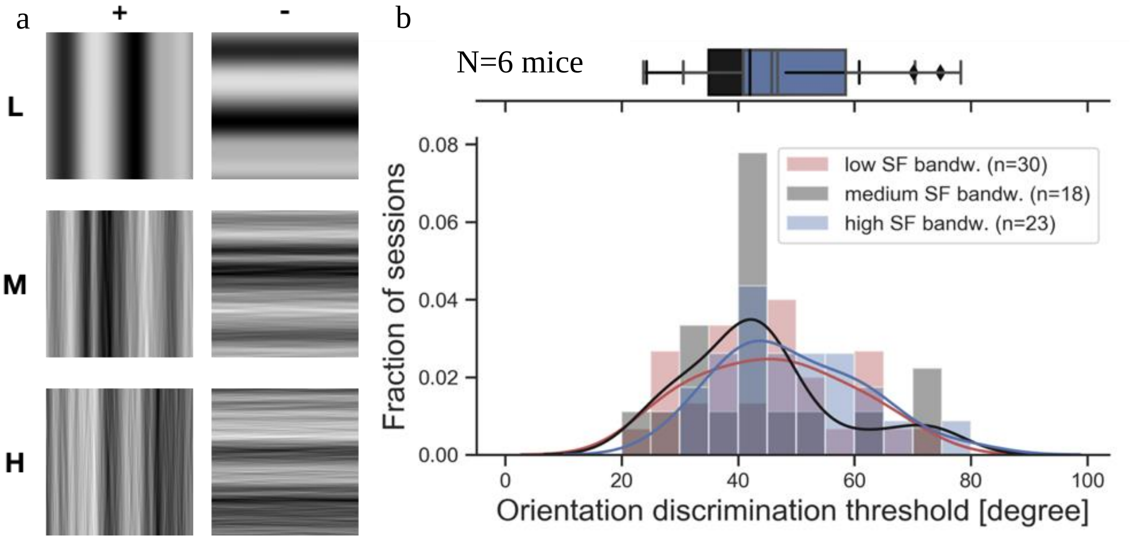


Figure 4.11: Distribution of orientation discrimination thresholds over different spatial frequency bandwidths.

a) Reward scheme of this experiment with example frames. The rewarded stimuli are marked with + and the disctractors are marked with -. On the left side the spatial frequency bandwidth is shown. They are at 0.004 cpd (L), 0.04 cpd (M) and 0.4 cpd (H).

b) Red-colored bars and lines represent the stimuli with a low spatial frequency bandwidth. Grey and black colored bars and lines represent the stimuli with a medium spatial frequency bandwidth, and blue bars and lines indicate the high spatial frequency bandwidth

The comparison in the orientation discrimination threshold (Figure 4.11a-b) showed the most prominent peak for medium spatial frequency bandwidth between 40° and 45° (indicated in grey bars and the black line). There is a drop in this distribution for higher thresholds shown by the mice. The stimulus with a low spatial frequency bandwidth showed a flat distribution with its maximum at 45°. There was no sharp decrease in the number of sessions with higher orientation discrimination thresholds. The shape of this distribution was similar to the orientation discrimination thresholds for the high spatial frequency bandwidth. Most of the sessions were counted for an orientation discrimination threshold between 40° and 45°. Overall, the medium spatial frequency bandwidth seemed to show a different trend than the other two spatial frequency bandwidths. However, this trend is not significant (Kruskal Wallis test=2.037; p=0.36).

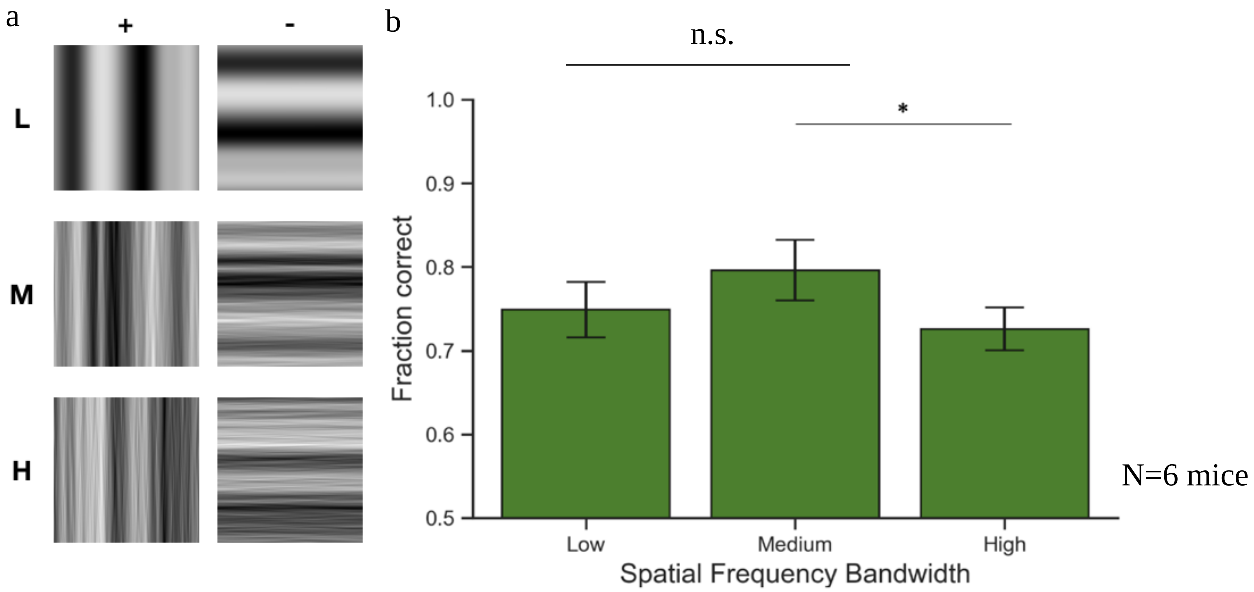


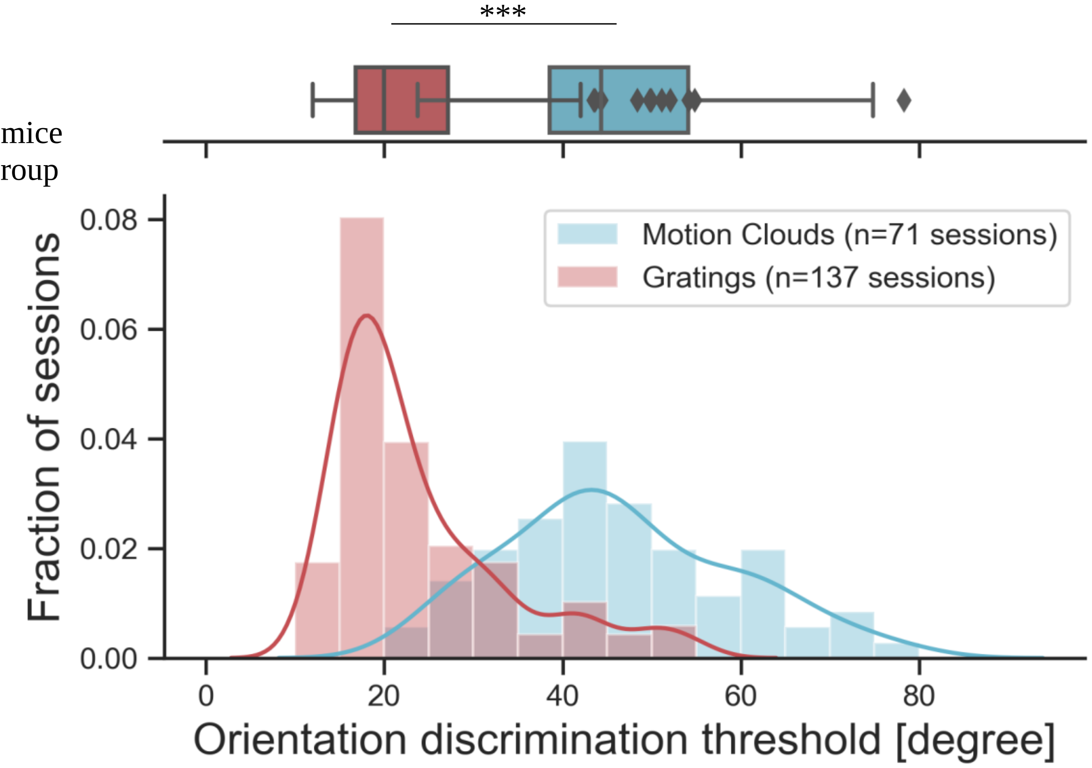
Figure 4.12: Fraction of correct trials in orientation discrimination over different spatial frequency bandwidths for orientation differences between 40° and 50°.

a) Reward scheme of this experiment with example frames. The rewarded stimuli are marked with + and the disctractors are marked with -. On the left side the spatial frequency bandwidth is shown. They are at 0.004 cpd (L), 0.04 cpd (M) and 0.4 cpd (H).

b) To have a dataset, which is more comparable to the spatial frequency discrimination task, with fixed differences in spatial frequencies, all trials were excluded which were recorded outside a window of an orientation difference from 40° to 50°. The comparison between a low and a medium spatial frequency bandwidth did not show a significant difference, while the mice were significantly worse in the orientation discrimination task when the stimuli had a high spatial frequency bandwidth compared to a medium spatial frequency bandwidth

In a comparison of the performance for the orientation differences between 40° and 50°, a slight increase of 5% on average is shown for the medium spatial frequency bandwidth

(Figure 4.12a-b). The animals reached overall a reliable discrimination performance in this specific orientation window. The difference between the three spatial frequency bandwidths is not significant (Kruskal Wallis test=4.914, p=0.08). A further groupwise analysis revealed a difference between the high and the medium spatial frequency bandwidth (U-Test=107.0, p=0.012)



N=6 mice
per group

Figure 4.13: Distributions of orientation discrimination thresholds for sine-wave gratings (red, see Chapter 3) and orientation discrimination thresholds in Motion Clouds (light blue). *Each session is analyzed for the specific orientation discrimination threshold. All orientation discrimination thresholds are shown as a histogram. For better visualization of the distribution, a boxplot is added on top, which represents the same set of data. In red, as a baseline, the data from the orientation discrimination task in Chapter 3 is used (red). The orientation discrimination data for motion clouds are pooled over the different spatial frequency bandwidth. The difference in orientation discrimination performance is significant.*

The comparison of the performance in the orientation discrimination task between Motion Clouds and sine-wave gratings (Figure 4.13), showed a difference in there discrimination performance. While the mice showed an orientation discrimination threshold between 15° and 20° for sine-wave gratings, there is a decline performance respectively an increase in the orientation discrimination threshold for the motion clouds (pooled over all three spatial frequency bandwidths). The difference is significant (U-Test=792.0; $p=2.2*10^{-23}$).

4.4.6. Summary

- Motion Clouds with a high spatial frequency bandwidth show a similar distribution as naturalistic images
- Wide-field imaging shows an increased activity for higher orientation bandwidths in Motion Clouds
- The optomotor response do differ over spatial frequencies, but not over different orientation bandwidths
- The orientation discrimination performance differed between a medium and a high spatial frequency bandwidth, without showing an overall effect. The orientation discrimination threshold between spatial frequency bandwidths is not significantly different.
- The spatial frequency discrimination shows significant differences between the high orientation bandwidth and the low and medium orientation bandwidth while performing in an active task.
- Sine-wave gratings are easier to discriminate for the animals than Motion Clouds in terms of orientation discrimination

4.5. Discussion

The experiments showed that there is a difference between passive viewing and an active task. While there was no difference observed in the optomotor response task, there was an increase in performance in the spatial frequency discrimination task when the orientation bandwidth increases. There was an unclear effect in terms of the spatial frequency bandwidth on the orientation discrimination performance. There was a slight difference in the performance between stimuli with a medium and a high spatial frequency bandwidth. There might be a discrimination optimum at the medium spatial frequency bandwidth of 0.04 cpd. This effect was not pronounced in the staircase experiment, so it is not possible to draw a clear conclusion.

4.5.1. Motion Clouds as a naturalistic stimulus

Motion Clouds are a suitable replacement for natural images. Natural images, even if they have on average distinct characteristics in terms of contrast-, spatial frequency- and orientation-distribution, are also characterized by a big variability. The stimulation with naturalistic images needs careful analysis of the specifically used images. The need for awareness even potentiates for naturalistic videos. This is crucial when the experimental design is similar to those studies, that aim for the description of neuronal activity to artificial stimuli. To interpret the results from naturalistic image evoked activity, it is easier to use a parameterized approach. Motion Clouds close the gap between the advantages of artificial images, like the strict orientation or spatial frequency without a bandwidth, and the disadvantage of a naturalistic image (Figure 4.6). It is possible to represent the distribution of an average naturalistic image in one stimulus. In this thesis, we were able to show, that there is a linear correlation in terms of spatial frequency distributions for motion clouds and naturalistic texture images.

4.5.2. Behavior Orientation discrimination

The spatial frequency does not have an obvious effect on the orientation discrimination performance. While there was no overall effect by the spatial frequency bandwidth, it was shown that there might be a small effect for a medium spatial frequency bandwidth for orientation differences between 40° and 50° (Figure 4.12). In addition, the animals showed an effect on performance for the motion cloud stimuli compared to sine-wave gratings, explainable by the orientation bandwidth. Most of the sessions resulted in an orientation discrimination threshold around 45°, which argues for the fact that the animals were still able to reliably distinguish between cardinal and oblique orientated stimuli.

This difference between motion cloud stimuli and sine waving gratings can be explained by the fact, that the motion cloud stimuli had an orientation bandwidth (Figure 4.13). Assuming an orientation bandwidth of 22.5°, there is already overlap between the two different orientated stimuli at an orientation difference of 45°. The orientation bandwidth in a motion cloud stimulus, adds a certain level of ambiguity in an orientation discrimination task. This is a helpful tool to control the difficulty level of a task to avoid performance levels close to the saturation of the psychometric curve. In this case, the orientation bandwidth of the motion cloud stimulus was helpful to modulate the overall difficulty of the task. When the task is too easy and the animals reach for any condition a high performance, it becomes impossible to compare different stimuli due to the orientation discrimination capability.

A medium spatial frequency bandwidth showed a non-significant trend to a more stable orientation discrimination performance. It can be argued that the high spatial frequency bandwidth also leads to a shift in the peak spatial frequency due to the arithmetic moment, but if this is the case, a significant difference between the low and the medium spatial frequency bandwidth could have been expected, but the difference of 5 % in performance between these two conditions did not show a significant difference. Assuming that the addition of a bandwidth recruited more neurons for evoked activity, the absence of a difference in the performance, leads to the conclusion, that the gain normalization hypothesis by Simoncini et al. 2012 can be supported in terms of orientation discrimination.

The general question of this topic, if more information in an enriched stimulus are helpful or not, can be answered for the spatial frequency discrimination. A higher orientation bandwidth led to a higher performance (Figure 4.10). In case of the orientation discrimination, we do not assume that there is an effect of the spatial frequency bandwidth. Following the multichannel hypothesis for sensory systems, it has to be concluded that orientations and spatial frequencies are computed by different channels, but it is unclear how they interact with each other. Earlier studies suggest that single neurons are tuned to specific orientations and spatial frequencies (DeAngelis et al. 1994), thus they can be distinguished into different sensory channels. Another study showed that if V1 neurons are optogenetically stimulated, the discriminability for orientation discrimination is enhanced (Pinto et al. 2013). From this finding, the idea arose that if more neurons are activated by a larger bandwidth of spatial frequency, the ability to discriminate orientations is increased. The present experiment can support this hypothesis with view on the increase of elevated responses at high orientation bandwidths. It has to be a part of the coming research, to investigate how many and which neurons are activated due to the increasing spatial frequency bandwidth. By now, the findings from the behavioral orientation discrimination experiment suggests a gain normalization, but it is not clear where this process happens. Candidates for gain normalization are the retina, the lateral geniculate nucleus or the primary visual cortex. Findings from macaque monkeys also suggested the processing of complex stimuli in a higher visual area (Freeman et al. 2013).

4.5.3. Spatial frequency imaging

The wide-field experiment showed that an increasing orientation bandwidth is linked to a higher evoked activity in the primary visual cortex for a spatial frequency of 0.04 cpd. The effect is absent for a spatial frequency of 0.16 cpd (Figure 4.9). First, the increased activity for the optimal spatial frequency of 0.04 cpd suggests that more neurons are recruited by the enriched stimulus. The fact that a stimulus, which contains more orientations also shows a higher overall activity of the primary visual cortex is contradictive to the previous finding for orientation discrimination in mice. While there was no behavioral effect for increasing bandwidths of spatial frequencies, there was a clear trend to higher activities in terms of orientation bandwidths. At a spatial frequency of 0.16 cpd, which is on the edge of the visible spectrum for a mouse (Roth et al. 2012) this effect was not present. This suggests that the stimulus, including the range of orientation information, was overall less well perceived. Nevertheless, the highest orientation bandwidth evoked the significantly highest activity in the primary visual cortex. Even though it has to be mentioned that wide-field imaging data does not lead to findings of the behavior of single neurons.

4.5.4. Spatial frequency behavior

The spatial frequency discrimination task revealed an increase in performance for a high orientation bandwidth compared to lower orientation bandwidths. Spatial frequency discrimination is a common task in mice (Prusky et al. 2000, Wang et al. 2016). Spatial frequency discrimination in naturalistic stimuli was not performed in mice so far, but in humans (Kauffmann et al. 2014, To et al. 2011). These studies were not based on parameterized naturalistic stimuli. The discrimination of naturalistic images, with respect to their structural similarity (SSIM), was done before. To decrease the SSIM a gaussian blur was used, which decreases the amount of high spatial frequencies (Yu et al. 2018). It was shown that a higher level of blurriness respective a lower overall spatial frequency made it easier for the animals to distinguish between the stimuli. This is also consistent with the Weber-Fechner-Law, which claims that discrimination tasks are easier on a low-intensity level. It remains unclear, which cues of the image the mice used to discriminate the images.

If the discrimination is based on single prominent structures, e.g. on the horizon between the landscape and the sky, the conclusions are not so clear to draw. This is another advantage of a nature-inspired parameterized and homogenous stimulus. Prominent structures, like a horizon, are completely excluded. Thus, the motion cloud stimulus is a powerful tool to investigate the visual system with a view on spatial frequency perception.

The behavior task is divided into a passive viewing task and an active viewing task. The optomotor response test, to quantify the passive perception of motion clouds did not show an effect of the orientation bandwidth (Figure 4.7). This might mean that other brain areas are stronger involved than in the active task. A candidate area for driving the passive viewing is the *superior colliculus*, which is linked to the eye movement. It can be speculated that parallel processing is less pronounced or absent with view on different orientation bandwidths in this area, but this question can not be answered clearly at this point.

Under the assumption that a passive viewing task, evokes less neuronal activity it can be concluded that higher activity is needed to utilize the advantage of a high spatial frequency bandwidth as shown in the active visual discrimination task. Compared to the results of the orientation discrimination task with increasing spatial frequency bandwidths the question remains, why there is an effect in one experiment, but not in the other. First, orientations and spatial frequencies have not the same tuning properties in the primary visual cortex. The lognormal distributed tuning of spatial frequencies is not symmetrical as the tuning for orientations. This could mean, that more neurons are activated for the low spatial frequency, which results in an overrepresentation over the high spatial frequencies. The quantitatively low number of neurons that are activated through the stimulation with high spatial frequency components of the stimulus might be not relevant for the task in this case. The orientation tuning in the primary visual cortex shows a normal distribution. Thus, if an orientation bandwidth is added, more neurons around the preferred orientation are activated. This might have a stronger impact on spatial frequency perception as vice versa.

4.5.5. Conclusions

Addressing the idea if a gain normalization neutralizes an effect through an enriched stimulus, it can be concluded that it seems to rely on the exact experimental design. Is there a task, where the animal is passively looking on a stimulus or is it actively engaged in a task? Is the sensory input, which the animal receives changing in contrast, spatial frequency or orientation? The present thesis can give the first insight into the perception of natural images of mice, with respect to objectively parameterized stimuli. It can be concluded, that this kind of stimuli is a valid way to investigate the mouse visual system. Since there is the difference between orientation and spatial frequency discrimination, it can be concluded, that there is a positive effect of different bandwidths in the stimulus, depending on the parameter, which has to be discriminated against. Therefore, following the title-giving hypothesis by Simoncini et al. 2012 “More is not always better”, this thesis can add “but in case of orientation bandwidth in a spatial frequency discrimination task, it is”. This does not argue in specific against the proposed gain normalization model, but it would add the idea that the tuning properties of specific channels also play a role. Since Simoncini and his colleagues focused their study on motion and spatial frequencies, this thesis is another stone in the natural perception-puzzle.

4.6. Outlook

The findings lead to a handful of open questions, but give the first insight into the natural perception of mice. Follow up experiments should focus on imaging approaches to quantify the neuronal activity in the primary visual cortex. This could support the idea of a gain normalization in the primary visual cortex for orientation discrimination tasks for varying spatial frequency bandwidths. The crux of the matter will be that head-restrained animals have to be engaged in a task. Therefore, the touchscreen chamber approach is not sufficient. Similar to the experiment on the oblique effect from the previous chapter, it is not clear, if it is possible to train an animal in the touchscreen chamber and transfer the task into a task for head-restrained animals. If the animal is not actively engaged in the task, it changes the results in a two-photon microscopy-approach.

Chapter 5: Teaching neuroscience with the touchscreen chamber

5.1. Abstract

The study of animal behavior is an essential part of neuroscience. Operant conditioning has made these studies available under laboratory conditions. Our current understanding how animals learn, decide and avoid is based on operant conditioning tasks. Furthermore, animal models of neurological diseases and drug screening of their treatments strongly rely on testing their behavior in standardized conditioning setups. Learning how these setups work and how these studies are designed, how animals are trained and studied during the task and how the obtained data is interpreted is indispensable for the successful training of neuroscience students. The participants were supposed to learn how to handle mice and perform simple experiments with them. Thus, the animals are trained by the participants to understand the basic principle of the touchscreen chamber, including habituation and touching the screen to respond to a stimulus. A diet restriction was introduced as well, which adds the factor of health scoring. The participants had the opportunity to train mice from the first habituation to the setup until a first experimental session including an orientation discrimination task, in which the animals had to distinguish between a horizontally and vertically orientated sine-wave grating. The animals showed a learning process which is analyzed by the students using modern tools, like the python programming language. In addition, the participants are guided to prepare their own translational experimental approach in PsychoPy. This experiment is then tested with all participants from the course to illustrate the different challenges of human and animal experiments. The feedback of the student showed the demand for courses that teach animal experiments in the context of modern methods and helpful tools. All in all, we can provide the concept of a practical course with a high impact on the education of undergraduates at a limited amount of costs.

5.2. Introduction

5.2.1. Why teaching Neuroscience with living animals?

The observation of animals and their behavior is an important point in neuroscience to link the understanding of the brain with the complexity of behavior. Operant conditioning is a method widely used in basic research but also in applied sciences like pharmacology. This practical course should give a basic understanding of these principles

The use of animals in research is still an object of lively debate (Rollin 2006, Fraser 1999, Ideland 2009). It is argued if there is still the need to use animals in research or if there is also the possibility to substitute them with a replacement. One proposed approach to replace animals is the use of computer models. A wide range of studies, where computer models replaced an experiment in the lab is based on molecular interactions in specific settings (Dewhurst et al. 1994, Knight et al. 2006). Compared to the simulation of a fully awake, behaving, higher mammal, this is a very limited number of variables, which has to be taken into account. To our knowledge, there is by now no computer simulation tool, which can simulate the complexity of a behaving animal. Another problem is, that a computer program can only be filled with known variables. This excludes the possibility to take unknown variables into account, without losing the explainability of the model.

For example, if the behavioral outcome is simulated with an artificial neuronal network, the outcome can be quite valid with respect to the input, but due to the computational process of an artificial neuronal network, it is impossible to draw conclusions about the specific process. This makes animal research in terms of behavioral experiments, for now, without alternative. Since there is still the need for behavioral experiments, it is of outstanding importance to teach behavioral experiments with a neuroscientific background to undergraduate students. This is not just following the goal to make them familiar with the handling of mice or the planning of an experiment. It can be assumed that undergraduate students are also sensitized to animal welfare by their first “hands-on” experience. Thus, it is helpful for the neuroscientific community to train future scientist, who knows how to work with animals, but also have a sense for the well-being of their research animals.

Learning objectives

1. A wide range of medical research is based on the screening of animal models. The interpretation of these studies requires a good understanding of used methods. The participants should receive first experiences with operant conditioning as a powerful tool to screen an animal’s behavior, with view on validity and variability of behavior
2. The participant should also learn how to plan an experiment. Therefore, they get time to realize a project from the first idea until the analysis
3. The participant should learn the basics of animal health monitoring
4. The participants should gain knowledge about the neuroscientific background of behavioral experiments and how this background is utilized to plan the experiment
5. The participants should collect their first experience with a computer-based experiment that requires python-programming on the beginner level
6. The participants should be able to apply their statistical and analytical skills to the collected data from the behavioral experiment
7. The participants should learn to present their results at the end of the course and collect them scientifically in a written report
8. The participants should learn about the role of animals in research, especially concerning mice. By the end of the course, they should be able to discuss animal experiments in terms of the 3R-principles

5.2.2. How feasible is the practical course?

To run the practical course a few prerequisites have to be fulfilled. An institute offering this course needs a place to keep mice or even better an own breeding facility. Then, a room is needed to place one or more touchscreen chambers as described in this thesis in Chapter 2. The costs for one box are kept low and it can be assembled easily. There is at least one supervisor needed to support the students with his or her programming knowledge in Python and PsychoPy (Peirce 2007, Peirce 2009). Besides the experimental apparatus and the animals, there no additional material or reagents needed. The used materials can also be varied due to the financial background of an institute. The number of animals is variable as well. We propose to plan one animal per participant. The course has to be a block course, which is held on consecutive days, excluding the weekends, over two weeks at least. This is including at least eight experimental sessions. It is possible, in accordance with the authorities to vary the research question of the practical course from time to time.

5.2.3. General concept of the course

The practical course is embedded in a module containing a weekly lecture (2h per week for 14 weeks) and a seminar of two days. The general ideas behind the research in behavioral neuroscience are included in the lecture, containing topics like learning and memory, psychophysics, reward and motivation, and attention. The seminar covers recent research from these fields. The practical course is open for students who participated successfully in the seminar and passed the exam on the lecture. The idea is to take concepts from the lecture and the seminar and apply them to the actual work in the laboratory. The practical course lasts for two weeks with 6 hours per day, including animal experiments, preparation of a human experiment and an introductory lecture. The practical course is concluded by the presentations of the participants.

5.2.4. Operant conditioning in an orientation discrimination task

The orientation discrimination task, described in chapter 3 of this thesis, is used for the practical course. According to the time able to prepare the course, the experiment can be performed with pretrained or naive animals. The pretraining is suited to show what steps are needed to bring an animal to the point to perform in a wished task, while the actual experiment delivers a dataset to create a psychometric curve. For the setting, where the participants receive naive animals it is more likely to show progress in the mice over the two weeks of the practical course. Operant conditioning is a widely taught principle in most of the biological studies, which include behavioral research. Therefore, it can be fitted into most curricula. The basic principle of the operant condition is described in the general introduction of this thesis.

We propose an orientation discrimination task, where mice have to distinguish between a horizontally and vertically orientated sine-wave grating. This experiment is performed abroad in several laboratories, mostly in a water maze apparatus (Prusky & Douglas 2000, Vermaercke et al. 2015). To test a hypothesis and test an experimental group and a control group the housing conditions are altered. Therefore, three animals are housed together in one home cage. As there were six participants in the practical course we tested six animals if there is an influence of enrichment in the cage. One group hat the possibility to exercise on a running wheel (n=3), while the other group did not have this opportunity (n=3). Different studies suggest the effects of voluntary exercise on behavioral tasks. One study shows a reduce path length and shorter latency for mice, which are exercising on a regular base on a running wheel in a spatial learning task (van Praag et al. 1999). Another study showed a later onset of these effects in mice aged between 16 and 18 months. In the practical course, it is possible to test for the question if young animals show effects as well at a relatively low cost.

5.2.5. Design of an experiment

To teach the basic principles of the design of a psychophysical experiment and programming we introduce the participants to the use of PsychoPy. PsychoPy is a stand-alone site package for Python. The main advantage of PsychoPy is that it is usable without any programming knowledge because there is a graphical user interface provided. This GUI is sufficient to build small experiments and provides an automatized creation of the python-code. This code can be modified, so the basic principles behind the python-syntax can be taught in a practical environment.

5.2.6. Time schedule of the course

The practical course lasted for two weeks. The first day started with an introductory lecture to repeat the basics of operant conditioning and different approaches in behavioral research. Besides that, the research question, in this case, the question if there is an effect of exercise on the performance in an orientation discrimination task. Afterwards, the software tool PsychoPy is introduced (see the following chapter “Design of an experiment”). After the introduction, the participants are introduced into animal handling and how to score the health of the mice. Mice are weighed daily to see weight development throughout the course and how they react to the food deprivation. The following days are dedicated to the training of the animals and the setup of an own experiment, which can be performed by the participants of the course. The last day of the practical course is planned for data analysis and preparing a short presentation to clarify the findings for the course for the written report.

5.3. Methods

5.3.1. Animals

The orientation discrimination task was performed in six male C57BL/6 mice, started with the experiments at an age of eight weeks. They were bred and kept at the RWTH Aachen University. The practical course was authorized in the license 84-02.05.40.17.055. During the experiment, the animals were kept on a turned Day/Night-cycle of 12h:12h. The animals were food-restricted outside the experiment and received an additional 2-3 g per mouse into the home cage. They received food *ad libitum* on one day of the week on which no experiments are performed. Per day they received at least 1.5 ml of water to maintain their wellbeing. They had access to water *ad libitum* all the time. It is also possible to conduct the practical course under a water restriction regime. The mice were weighed and observed before the experiments start. The animals were individually scored for deviant behavior to check for stress symptoms, injuries or other relevant symptoms. This included the activity of the animal, the movement, the breathing frequency and skin turgor. Minor impairments were scored with one point, major impairments are with two points. If there were no major impairments observed, the animal had to be monitored carefully if the cumulative score was between one and four. At a cumulative score of 5 or higher or a major impairment the animal had to be removed from the experiment and had to be euthanized. At no point of the experiment a euthanasia was conducted. One group of three animals was housed with a running wheel, while another group of three animals was kept without a running wheel.

5.3.2. Behavioral Apparatus

The python-based touchscreen chamber (described in Chapter 2) was used for the animal experiments in the practical course (Figure 5.1). Briefly, the trapezoidal-shaped operant conditioning chamber utilized an infrared touchscreen frame for a direct response to the stimulus. After a correct response, a low-pitched tone is played by a loudspeaker and a green LED indicates the availability of a reward. An incorrect response results in a high-pitched tone and a bright screen as a light punishment.

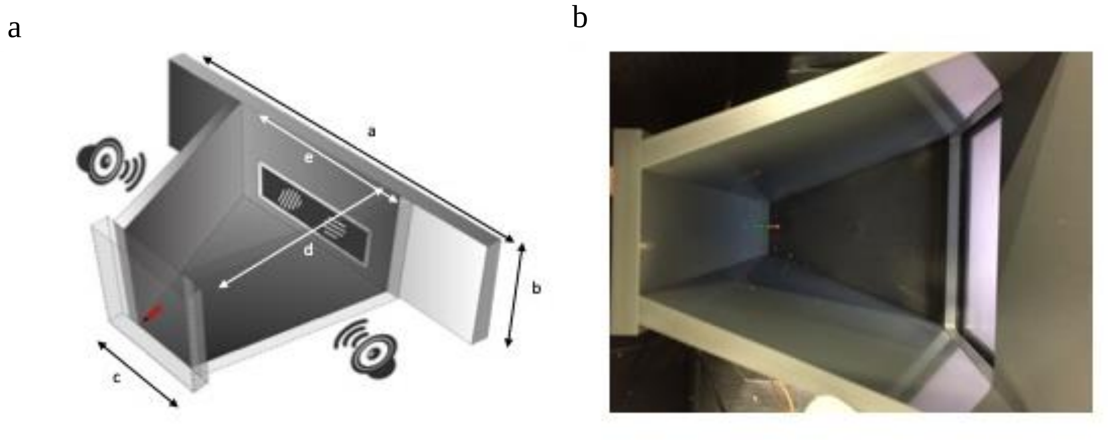


Figure 5.1: Python-based touchscreen chamber

a) Schematic view including the assembled polyvinyl chloride plated, the screen and the loudspeaker

b) Photograph taken from above

The experimental scheme is equal to the visual discrimination task with a cardinal target as described in chapter 3:

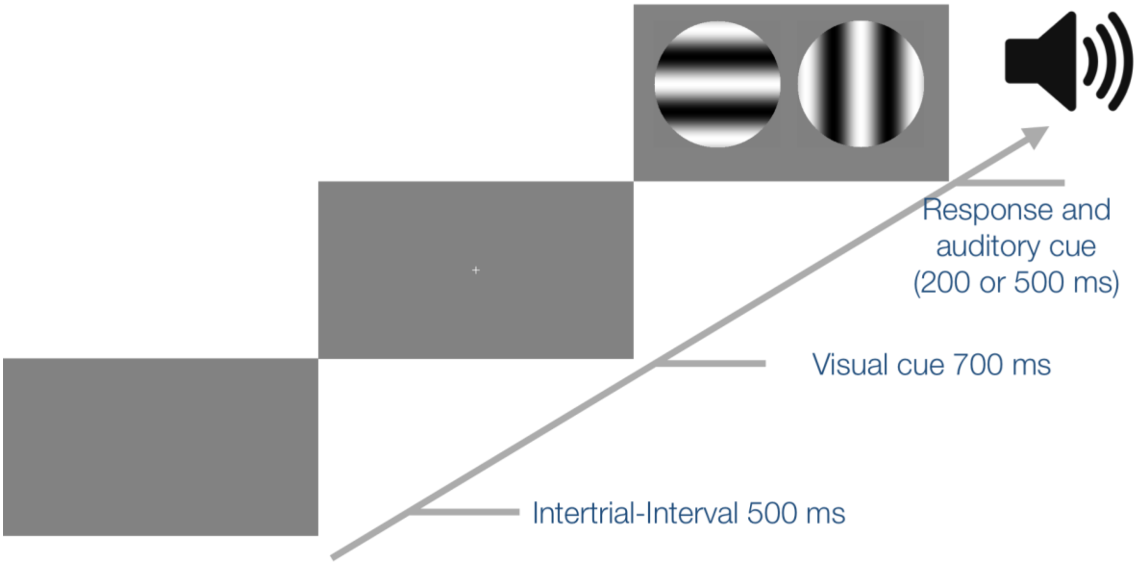


Figure 5.2: Scheme of the visual discrimination task with a cardinal target. *The trial is started by an intertrial-interval of 500 ms, followed by a visual cue for 700 ms. Afterward, the stimuli are presented. In this paradigm, the horizontally orientated sine-wave grating is the target, while the vertically orientated sine-wave grating is the distractor. According to the response, the auditory cue is short (200 ms) or long (500 ms)*

Every single trial started with an intertrial-interval of 500 ms (Figure 5.2). Afterward a visual cue, a white cross was shown on the screen for 700 ms. Then, two sine-wave gratings are presented. One of them is orientated horizontally (S+) and one is vertically orientated (S-). The response to the stimuli is followed by auditory feedback of 200 ms (if the response was correct) or 500 ms (if the response was incorrect).

5.3.3. Animal Handling

On the first day, the participants are asked to collect their first handling experience. Therefore, the mice were observed at first in their home cage. It should be explained how to observe the animals to see characteristic stress markers. The specific markers were described in Chapter 2 of this thesis. After that, the participants were asked to put their hands quietly in the home cage, so the mice can get used to human hands. To keep the animal handling to an extent, where animals are not stressed, the participants are asked to grab a mouse by the tail and put them onto their hand. This was also the handling procedure for placing the mouse into the touchscreen chamber.

5.3.4. Pretraining Habituation

Three days before the first pretraining, the food restriction was introduced. When the animals did not show a health score above 1, the habituation procedure was started. In the habituation phase, each animal was placed in the touchscreen chamber for twenty minutes. At this point, the script “phase0.py” was executed. The animal received rewards by activating the lick detection. It was indicated by a green light that a reward is available. Every activation of the lick detection was followed by a short timeout of five seconds. This was done to avoid that the animal activates the lick detection constantly. The animals proceeded to the next phase after they reached the criterion when they collect one reward per minute on two consecutive days.

5.3.5. Pretraining Touchscreen Training

After the animals reached this phase, they need to learn to touch the screen in order to receive a reward. Therefore, the animals were placed in the touchscreen chamber for 45 minutes on the first day, 30 minutes on the second day and 20 minutes on each following day. After the animals touch the screen, a low pitched tone was played via the loudspeaker and the green LED turned on. The green LED indicated that there is a reward available to collect. After the animal collected the reward, there was no further timeout and the animal could touch the screen again. When the mice collected more than one reward per minute on two consecutive days, they proceeded to the experimental training.

5.3.6. Orientation discrimination

The orientation discrimination task was started after the animals fulfilled the pretraining criterions successfully. A trial started with a grey screen (ITI) of 500 ms. Afterward, a white cross was shown as a visual cue to indicate the start of the response period. A horizontally orientated sine-wave grating (S+) is shown next to a vertically orientated sine-wave grating (S-) with an equal spatial frequency and phase. The mouse is therefore placed in the touchscreen chamber and the script “VD cardinal.py” is started. The animal can perform in a maximum of 100 trials, which is equal to 20 to 30 minutes for one experimental session in one animal.

5.4. Results

5.4.1. Habituation to the touchscreen chamber

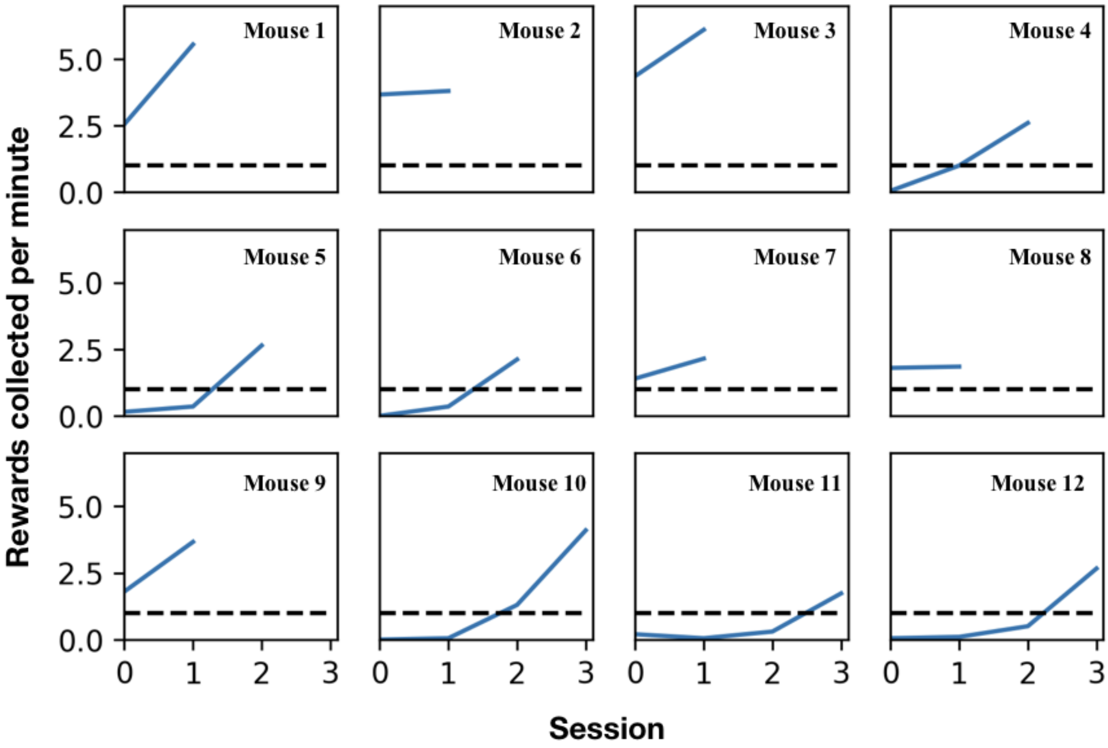


Figure 5.3: Rewards collected per minute during the habituation phase *Every blue trace represents the collected rewards per minute by an individual mouse. To collect a reward the mice had to touch the water spout when the green light is activated. The black dashed line indicates the threshold, which needed to be passed on two consecutive days to surpass the next stage of pretraining. One session was performed per day.*

Figure 5.3 shows the different amounts of collected rewards per minute for each individual mouse. Fifty percent of the mice started with a number of rewards collected above the threshold in the first session. Every mouse, except mouse 8 showed a positive trend toward more rewards collected per minute. To keep the time schedule of the practical course, not every animal reached the criterion on two consecutive days before proceeding to the next phase. These animals are mice 5, 6, 11 and 12. In general, it can be observed that some animals already started at a high level (mouse 1, 2 and 3), while other mice started close to zero collected rewards per minute (mouse 4, 5,6,10,11 and 12).

5.4.2. Learn to touch the screen

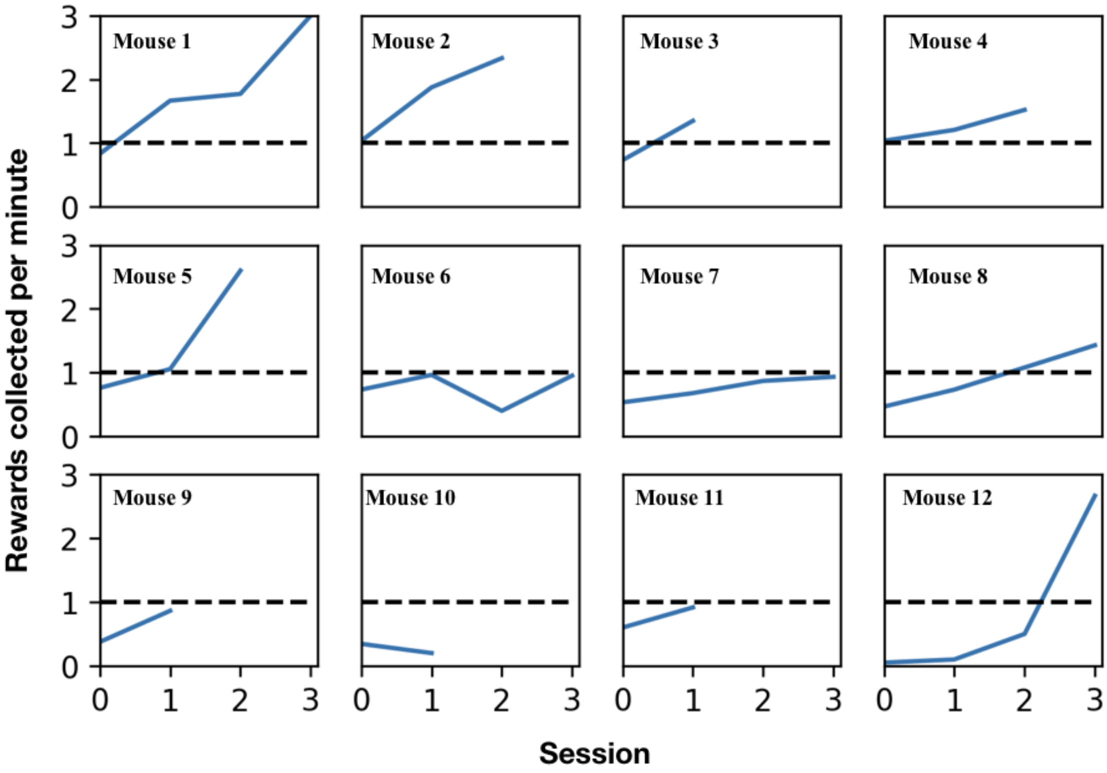


Figure 5.4: Rewards collected per minute, when the mice have to touch the touchscreen in order to collect the reward. *Every trace represents the trials per minute, which a mouse completed over several sessions. One session per day was performed. The black dashed line indicates the threshold, which needed to be passed in order to proceed to the experimental training.*

Figure 5.4 shows the rewards collected per minute when the mice had to touch the screen first in order to activate the lick detection. Most of the mice showed a positive trend for consecutive sessions, except mouse 6 and mouse 10. Mouse 1, Mouse 2, Mouse 4 and Mouse 5 reached the criterion of two consecutive days with one or more collected rewards per minute. Mouse 3 showed a larger number of rewards on the second day and did not reach the criterion as well as mouse 12, which passed the threshold on the fourth day. Mouse 3, mouse 9, mouse 10 and mouse 11 only performed in two days due to organizational reasons. To show the orientation discrimination task within the practical course, all mice were tested in the task on the last day.

5.4.3. Orientation discrimination

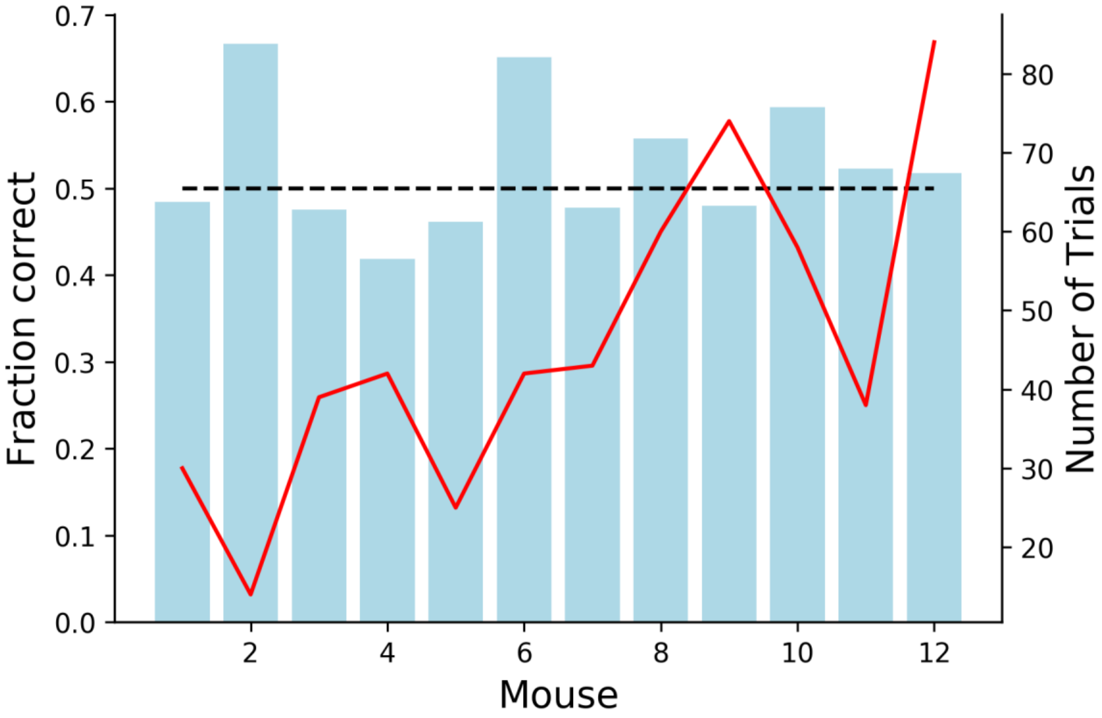


Figure 5.5: Fraction correct in one session for different mice (light blue bars) and the number of trials performed in this session (red line). *The black dashed line indicates the guess rate for the fraction correct-axis for each respective mouse. Every bar represents one mouse.*

Figure 5.5 illustrates the fraction of correct trials, indicated by the blue bar plots and the left y-axis. The guessing rate is at a fraction of 0.5 of all trials within the session. The red solid line indicates the number of trials. The data is shown for every individual mouse. As a part of the practical course, the data can be linked with the pretraining data. None of the mice showed a performance significantly above or under the guess rate (binomial test, n=trials, p=0.5, performed for each individual mouse, see Table 5). Mouse 1 for example, who performed well in the pretraining and learned fast that it has to touch the screen, showed just a relatively low number of trials in the first session of the orientation discrimination task. Mouse 12, which needed more sessions than others to collect a stable number of rewards in the habituation phase and also had late learning of touching the screen, showed a very high number of trials in the orientation discrimination task. Overall, the lowest number of trials was 14, performed by Mouse 2. The highest amount of trials was done by Mouse 12, which performed in 84 trials.

Table 5: Trials and performance in the orientation discrimination task for each mouse. *The p-value indicates if there is a difference between the performance and the guess rate of 0.5, tested with the binomial test*

Mouse ID	Trials	Performance	p-value
#1	30	0.48	0.86
#2	14	0.67	0.42
#3	39	0.48	0.75
#4	42	0.42	0.28
#5	25	0.46	0.69
#6	42	0.65	0.09
#7	43	0.48	0.76
#8	60	0.56	0.52
#9	74	0.48	0.73
#10	58	0.59	0.23
#11	38	0.52	1.0
#12	84	0.52	0.91

5.4.4. Participants feedback

The participants were asked for feedback. They provide it anonymously as a part of the teaching evaluation at the RWTH Aachen University. Five out of 12 students replied to the evaluation. They graded the course according to an internal grading system (1=best, 5=worst). The general concept of the course was graded with 1.5. Most students quantified the time spend on the preparation and follow up work with three to five hours. Similar to the grading, they had to mention how strong they agree with different statements (1=strongly agree, 5= strongly disagree). They were asked, to mention if they were able to apply what they learned during the lecture in the practical course and strongly agreed (1.6) and if the practical course improved their experimental skills (1.4). All participants classified the supervised work with mice as “very important”.

In addition, they provided feedback on the course:

“I liked the fact that students were free to apply their creative thinking on the design of an experiment.”

“[I liked] working with mice and [the] insight into animal experimental setup [and] handling”

“[I liked the] possibility to work with mice; (in my opinion) the learning effect could not have been achieved by showing videos or simulations”

“good insight into the “everyday life” of a behavioral scientist (from data acquisition to analysis)”

5.5. Discussion

Operant conditioning is a widely used principle to test different behavioral phenotypes. While the water maze task plays a big role in basic and applied research, the high throughput of automatized touchscreen chamber experiments is described in a wide range of studies (Brigman et al. 2009, Delotterie et al. 2015, Fisher et al. 2019). To educate students to make a reasonable choice on their methods when they are in the position to decide, we think it is more than important to provide experience in modern methods of a field. To give a comprehensive overview of the use of the touchscreen chamber with respect to the pretraining as well as the proper use of PsychoPy, a framework is provided to conduct a whole psychophysical experiment from the planning to the data analysis. When the number of mice is large enough, it is possible to show examples for fast, slow and mediocre learners. This also gives an impression of the challenges of behavioral experiments.

A practical course with already pretrained mice gives the opportunity to record full psychometric curves. This can give another aspect to the analysis of the experimental data, but also demands a higher level of preparation. The pretraining of 12 mice takes roughly three weeks and six hours per day to reach a level where the animals show a stable performance in the practical course. In this point the design of the practical course is restricted to the capacities of a facility, especially if the mice can be reliably trained every day. It has to be considered that an extensive pretraining is no guarantee for stable results. The animals have to get used to the participants and are likely to show a high variability. The advantage of a pretraining by the participants is a steady progress, since the animals do not need to much time to proceed to the next training stages. For a stable orientation discrimination, the animals need normally between 9 and 15 days of training. It is likely that a group of animals contains slow learners and stuck on the guess rate for a time, which extends the practical course.

In general, the practical course can be realized with a relatively low amount of money. One touchscreen chamber can be built for around 700 €. The supervision requires some knowledge in the use of Python or better in PsychoPy as well as profound experience in animal handling. In addition, participants can also be taught in the basics of deep-learning-based video analysis, depending on the specific time schedule. Therefore, videos can be recorded in the touchscreen chamber. The analysis with DeepLabCut (Mathis et al. 2018) can be included, even if it needs more time for the preparation. Overall, we can conclude that the practical course is a good way to teach the basic principles of behavioral testing, data analysis and applied animal welfare, which is shared by the participants. The feedback we got for the practical course is overall very positive and shows the demand for experiments with living animals as a part of the curriculum in the education of young neuroscientists.

Chapter 6: General Discussion

The main question of this thesis was the transition of well-known behavioral paradigms using an automatized and flexible approach as well as the transition from artificial stimuli to nature inspired images. The idea was to gain a deeper understanding of how the brain is processing these nature inspired stimuli by a functional readout. Therefore, we studied the behavior of mice with view on their perception of enriched stimuli connected to cortical activity. The flexible approach of a python-based touchscreen chamber opened the gate to investigate the effect of the orientation tuning in the mouse visual system and turned out to be a method, which also helps to educate students in the field of behavioral neuroscience.

At the beginning of the thesis, four goals were postulated:

1) Provide a flexible and comprehensive touchscreen chamber for operant conditioning

The present thesis showed how to build a comprehensive touchscreen chamber with an open-source character at a maximum flexibility. Compared to other approaches, we can conclude that we presented a touchscreen chamber for operant conditioning which is capable to fulfill all modern requirements without the burden of restrictive software. Due to unlimited access to the software and manuals for the hardware, we see a high relevance for the approach of open science. The touchscreen chamber was tested with view on the performance of mice in pretraining steps as well as in experimental training. The performance was comparable to other approaches in operant conditioning. Compared to rather simple approaches using iPads and Arduinos, we can provide here a high functionality, but the price is higher for our touchscreen chamber compared to O´Leary et. al 2018, Pineno 2013 and Leising et al. 2013. Nevertheless, we have to conclude that the demand always depends on the specific scientific question, so less flexibility is not a disadvantage per se. In our approach the animals did not need more time to understand the basic principle of the touchscreen chamber. They acquire the ability to collect water in the touchscreen chamber within a low number of sessions. Individual differences showed that some animals also learned very fast, when they had to touch the screen in order to get rewarded. On the other hand, some animals needed more sessions to learn that step. After the animals learned this step, they started the experimental training on an orientation discrimination task. The tested animals learned the orientation discrimination task within 9 to 15 sessions, which is on the same level as the Bussey-Saksida-Touchscreen Chamber (Bussey et al. 2008). In principle, the touchscreen technology can be compared to other ways to respond to a stimulus. In other settings the response via a lever as in the classical Skinner-Box or the response via a nosepoke or a lick (Musall et al. 2019, Zoccolan et al. 2009) is a helpful approach, concerning if the mouse should move or not. Not moving, head-restrained animals gives us as researchers the possibility to collect data via in vivo imaging and electrophysiology. With view on the timescale of these experiments, the need for a surgery and a more sophisticated habituation to the setup, expands the demand of time. Thus, a touchscreen chamber opens the door for a high throughput of behavioral experiments with the tradeoff that in vivo recordings of the brain a harder to achieve. Mobile implants are also described (Juavinett et al. 2019) and they deliver a bridge between freely moving mice and the recording of brain data in vivo. This gives the possibility to uncouple the collection of a reward from the response to the stimulus. This would give the possibility to divide the recordings of the brains behavior to sensory input from neuronal activity during the collection of the reward. All in all, the touchscreen chamber which is presented in this thesis provides a very flexible framework for operant conditioning. Due to the use of open-source python software, the experimental paradigms can be easily adapted to other experimental questions. As presented, the orientation discrimination task was also rearranged into a staircase procedure, which gave an insight into the basic perception of orientations. This level of flexibility is a big advantage with view on translational experiments, since the experiments can also be adapted easily in a test for the human visual system. All in all, we provide a comprehensive framework for behavioral experiments in rodents. Further applications and the perspective is described in the general outlook.

2) Test the functional effect of the overrepresentation of neurons tuned to cardinal orientations

To proof the functionality of the touchscreen chamber, we addressed the question if there is behavioral relevance of the fact, that there is an overrepresentation of cardinal orientated neurons in the primary visual cortex of mice. We were able to demonstrate with the newly created python-based touchscreen chamber, that the so-called oblique effect can be masked with an early onset of training, which emphasizes the underrepresented orientations. In contrast to that, there is a big behavioral influence if natural tuning is emphasized in the training. This hints that mice are able to adapt to meaningful stimuli against the natural properties of their primary visual cortex and connected brain areas, like the posteromedial cortex.

To test this, a parallel visual discrimination task was performed by four mice. In this experiment, the animals are trained on two different sets of stimuli with two different targets and two different distractors. It was clearly shown, that a horizontal target was easier to distinguish then an oblique-orientated target. This led to the question, how different the orientation discrimination thresholds are for different orientated targets. In an experimental approach where it was tested, how mice are performing after a retraining, it was shown that the orientation discrimination performance is much worse for an oblique orientated target. This applied when the mice were trained on a horizontal target before. We conclude that the initial training reinforced the natural tuning properties of neurons in the visual system. The retraining was successful when a mouse learned a turned reward scheme at a difference of 45°. To measure the effect of the initial training, another batch of mice was trained on an oblique orientated target from the first experimental training session. Against the expectations, the mice showed the same performance as they would have been trained on a horizontal target. Without an additional imaging experiment, to measure the activity of single cells, it is not possible to draw a clear conclusion, but changes in the neuronal activity within the primary visual cortex are reported. Overtrained animals showed the effect that the number of cells, which are not orientation selective is decreased in an orientation discrimination task (Poort et al. 2015

3) Test the perception of natural-inspired stimuli in the touchscreen chamber

The transition to nature-inspired stimuli was successful in terms of orientation and spatial frequency discrimination. The animals perceived the stimuli well enough to perform in those tasks, which was not shown in Motion Clouds before. This was a crucial step for the investigation of nature-inspired stimuli in mice with view on the motion clouds. The link to the wide-field imaging approach gives a more meaningful insight, into how nature-inspired stimuli are perceived. It has to be mentioned that there is no comprehensive insight into which brain regions in the mouse are involved in this process yet. It was shown that the bandwidth of orientation can play a role in the perception of other statistical parameters. From the experiments in this thesis it can be concluded that the discrimination of spatial frequencies relies on the bandwidth of orientation, but we were not able to show a clear effect vice versa. The orientation discrimination was not clearly affected by the spatial frequency bandwidth. One reason for this effect could be the different general tuning of the visual system in terms of orientations and spatial frequencies. While the orientation tuning follows a gaussian distribution with the highest tuning for cardinal orientations, the spatial frequency tuning follows a log-normal distribution. The latter emphasizes low spatial frequencies more than high spatial frequencies. The described effect is pronounced in the discrimination task, but not in the optomotor response. This argues for a significance of different behavioral states, since the optomotor response test did not require active behavior.

4) Utilize the touchscreen chamber to teach neuroscience

The method of the touchscreen chamber as a teaching subject for operant conditioning in mice was presented in this thesis. In the practical course, it was possible to collect relevant data to teach the basic principles of animal training in neuroscience. The pretraining procedures demonstrated a comprehensive insight into a visual discrimination experiment, as it is used in behavioral neuroscience. From the students feedback, we can conclude that there was a demand for practical courses which are teaching the normal routine of a researcher in the field of behavioral experiments. It was shown that the animals show, especially in the pretraining phases a steady progress to illustrate the steps, which are needed to reinforce a desired behavior. We were able to show, that the animals can be trained to the stage, where they start to perform in a visual discrimination task within two weeks, even if the “experimenters” are not experts at animal training. A psychometric curve, as a central analysis outcome, was not achieved due to the limited time. One option is to conduct the practical course with animals, which are already pretrained. This approach needs a higher preparation time. From the other point of view, the translational approach, to teach students on a psychophysicists tool like PsychoPy, was also well received. All in all, the practical course can be concluded as a success, taking the students feedback into account.

Chapter 7: General Outlook

The experiments in this thesis answered a lot of questions asked in the beginning, but there a still open and new questions. The touchscreen chamber provides a flexible open-source framework for a wide range of experiments concerning behavioral phenotyping and basic research on the sensory systems. The flexible character opens several doors into more research questions. The touchscreen chamber could be integrated into a bigger infrastructure. A collection of standardized datasets, comparable to MouseBytes (Beraldo et al. 2019) could be implemented. This would also support translational approaches for the screening of different behavioral phenotypes. Therefore, more paradigms need to be developed. The software used in this thesis provides a stable environment for further steps. Concrete next steps would be to establish the method in other laboratories as well. Establishing a costumized behavioral apparatus has, next to several advantages also its downsides. One the hand there is no company in the background, which could provide an extensive service and guarantees. To repair malfunctions during a running experiment can be a very crucial point, especially in learning tasks. Therefore, there is the demand that several members of a group are familiar enough with the setup to do a regular service to avoid downtimes due to malfunctions. Once the hurdles are cleared, the touchscreen chamber is a flexible approach to achieve a high throughput of behavioral experiments. This also leads to a higher quality of experimental data, since there are mice which are not learning well. Due to the high throughput it is possible, to identify and exclude these mice after several sessions, but we were not able to identify an early marker for bad learners.

The functional effect of the overrepresentation of neurons tuned for cardinal orientations was shown in several animals in two distinct behavioral experiments. Another piece of the puzzle would be electrophysiological or imaging data straight from the primary visual cortex. Therefore, we want to recommend an experiment, which measures the orientation selectivity before the training starts. After an extensive training on a certain stimulus, it has to be checked in another imaging experiment if and how the orientation tuning changed. Even if there is the clear evidence, that there is a change on the functional level, it has to be validated in the future to draw clear conclusions. The established staircase procedure could be a helpful addition to the screening with view on different mouse models. The visual perception is in a large number of neurodegenerative diseases impaired (Uc et al. 2005, Gomez-Tartosa et al. 1996, Kurylo et al. 1996) and the staircase procedure provides a way for a screening on a finer scale. The influence of retraining needs a deeper investigation. In this study, it was only possible to retrain two animals on a different reward scheme for the staircase experiment. This leaves space for discussion and criticism. It is possible that these two animals learned a new reward scheme, but were not able to transfer it to a task with a dynamic level of difficulty. It could be possible that these two animals relied on the discrimination between patterns, but did not actually learn to discriminate between orientations properly. Nevertheless, the stronger point of this experiment is, that we were able to train four mice to detect an obliquely orientated target, which marks the way to the question, how the orientation tuning in the primary visual cortex may change and influences the behavior with view on the functional output.

The use of Motion Clouds in the investigation of nature inspired stimuli in mice could be a future topic. First, the capability of mice to perceive motion clouds in principle was a crucial step. The big advantage of Motion Clouds over natural photographs is the standardized character. There are consistent image statistics over the whole realization, while photographs or natural videos contains characteristic structures like horizons, which may lead to different activation patterns in the visual system. Another problem for discrimination experiments is, that these characteristic structures could lead the decision instead of the desired parameters of the image. Besides the big chances for behavioral experiments to collect functional evidence for how natural perception works, the results should be supported by electrophysiological or imaging experiments for single cell activity. This could lead to a deeper understanding of the neuronal computation of images with more complex statistics. Since there are approaches to explain the computation with gain normalization (Simoncini et al. 2012), this could be tested further in mice. This could give a translational insight into this topic, assuming that there are similarities between the computation in mice and humans. In general, the field of natural perception is still asking the question, how a natural stimulus exactly should look like. Indeed, a MotionCloud does not give the impression of being a tree in the forest and the search for a perfect natural stimulus is rather a philosophical question. We argue here for the MotionCloud with view on the high level of parameterization, while we accept the tradeoff of a stimulus, which does not look like any natural scenery for the sake of natural image statistics. When it comes to a natural perception, for most of species not just the visual system place a role. Contextual perception of visual input, odors and acoustic input also play a big role to evaluate a certain situation. The framework around the Touchscreen Chamber as well as the experience from the perception of Motion Clouds can lead to a wide range of questions concerning the multisensory integration. How is a visual task learned, when it is linked to an acoustic signal? Are these sensory modalities competitive to each other? This questions can be investigated with the finding of this thesis.

All in all, this thesis provides a further step in the investigation of the principle understanding of the visual system with view on several questions, but every answered question leads to a number of new questions, which need to be answered in the future.

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Curriculum Vitae

Personal Information

Name: Christopher Wiesbrock
Adress: Rosenbenden 12, 52511 Geilenkirchen
Telephone: 0160/90234104
Email: christopher.wiesbrock@rwth-aachen.de
Date of Birth: 04.12.1987
Nationality: German

Education

Realschule Heepen, Bielefeld	08/1999-07/2004
Ceciliengymnasium, Bielefeld	08/2004-06/2007
Rudolf-Rempel Berufskolleg, Bielefeld	08/2008-06/2010
Bielefeld University	10/2010-09/2014
RWTH Aachen University	10/2014-01/2017

Qualification

Abitur	06/2007
Management Assistant for Wholesale and Foreign Trade (IHK)	06/2010

Bachelor of Science

Biology, profile: “Behavior and neuronal mechanisms” Title of thesis: Effects of non-invasive corticosterone manipulations on the mating behavior of zebra finches	09/2014

Master of Science

Biology, profile: “Biological information processing” Title of thesis: Touchscreen-based orientation discrimination in nature-inspired stimuli	01/2017

Promotion (Dr. rer. nat.)	06/2020 (expected)

Work experience

Apprenticeship as Management Assistant for Wholesale and Foreign Trade at ek/servicegroup, Bielefeld

08/2008 - 06/2010

Employee at the department for public relations at ek/servicegroup, Bielefeld

07/2010 - 09/2010

Student assistant in the Department of Neurophysiology, RWTH Aachen

03/2015 - 12/2015

PhD student in the Department of Neurophysiology (Supervisor: Prof. Dr. Kampa), RWTH Aachen

01/2017 - 01/2020

Relevant teaching experience

- Supervision of Bachelor- und Mastertheses in the field of Behavioral Neuroscience
- Planning and supervision of the practical course „Behavioral Neuroscience“
- Planning and supervision of the practical course „Data Analysis and Statistics“ in the Research Training Group „Multisenses – Multiscales“

Computerskills

- Microsoft Office (advanced)
- Python (advanced)
- SPSS (advanced)
- Matlab (advanced)
- Tensorflow (basic)
- R (basic)
- HTML (basic)

Languages

- German (native)
- Englisch
- Spanish (basic)

Conference contributions

- Poster presentation “Orientation discrimination in mice examined with a novel flexible touchscreen chamber reveals cardinal preference over oblique orientations, NWG Göttingen 2019
- Abstract “The impact of coloration on the predation risk of subadult Fire salamanders (Salamandra salamandra)”, DZG 2013