NEURONAL AND BEHAVIORAL MECHANISMS OF EXTINCTION LEARNING AND RENEWAL

by

Sarah Starosta

A thesis submitted in partial fulfilment of the requirements for the degree of

Philosophiae Doctoris (PhD) in Neuroscience

from the International Graduate School of Neuroscience

Ruhr University Bochum

March 17th 2015

This research was conducted at the Department of Biopsychology, within the Faculty of Psychology at the Ruhr University under the supervision of Prof. Dr. Onur Güntürkün

Printed with the permission of the International Graduate School of Neuroscience, Ruhr University Bochum
**Statement**

I certify herewith that the dissertation included here was completed and written independently by me and without outside assistance. References to the work and theories of others have been cited and acknowledged completely and correctly. The “Guidelines for Good Scientific Practice” according to § 9, S 3 of the PhD regulations of the International Graduate School of Neuroscience were adhered to. This work has never been submitted in this, or a similar form, at this or any other domestic or foreign institution of higher learning as a dissertation.

The abovementioned statement was made as a solemn declaration. I conscientiously believe and state it to be true and declare that it is of the same legal significance and value as if it were made under oath.

Sarah Starosta

Bochum, 17.03.2015
PhD Commission

Chair:

1st Internal Examiner: Prof. Dr.

2nd Internal Examiner: Prof. Dr.

External Examiner:

Non-Specialist:

Date of Final Examination:

PhD Grade Assigned:
Nothing in neurophysiology makes sense except in the light of behavior
Table of contents

1. Introduction .................................................................................................................. 1

1.1. Learning on a behavioral level ............................................................................... 1

1.1.1. Classical and operant conditioning ................................................................. 2

1.1.2. Acquisition and extinction learning ............................................................... 3

1.1.3. Appetitive and aversive unconditioned stimuli .............................................. 4

1.1.4. Context-specificity of learning ....................................................................... 5

1.2. Neuronal mechanisms of learning ...................................................................... 6

1.2.1. Acquisition vs. extinction and appetitive vs. aversive outcomes – distinct or similar mechanisms? ................................................................. 6

1.2.2. A neuronal network for adapting behavior to the environment ............... 8

1.3. Comparative considerations .................................................................................. 10

1.3.1. Birds in neuroscientific research .................................................................. 10

1.3.2. Avian and mammalian brains ........................................................................ 11

2. Influence of expectancy violation on context-specificity of acquisition and extinction in a appetitive sign-tracking task ......................................................... 14

2.1. Introduction .......................................................................................................... 14

2.2. Materials and Methods ....................................................................................... 17

2.2.1. Subjects .......................................................................................................... 17

2.2.2. Behavioral apparatus ...................................................................................... 18

2.2.3. Procedure ........................................................................................................ 18

2.3. Results .................................................................................................................. 21

2.3.1. Phase 1/ Acquisition ....................................................................................... 21

2.3.2. Phase 2/ Extinction ......................................................................................... 22

2.3.3. Phase 3/ Retrieval ........................................................................................... 23

2.3.4. Difference in responding between contexts ................................................. 25

2.3.5. Control stimuli ................................................................................................. 26

2.3.6. Latent inhibition ............................................................................................... 27
2.4. Discussion..................................................................................................................................28
   2.4.1. Comparison of acquired and extinguished responses.........................................................28
   2.4.2. Influence of expectancy violation .........................................................................................30
   2.4.3. Summary................................................................................................................................31
3. Context-specificity of acquisition and extinction in a within-subject design..................................................32
   3.1. Introduction ..............................................................................................................................32
   3.2. Materials and Methods ............................................................................................................33
       3.2.1. Subjects ..............................................................................................................................33
       3.2.2. Behavioral apparatus .........................................................................................................33
       3.2.3. Procedure ..........................................................................................................................33
       3.2.4. Analyses ............................................................................................................................35
   3.3. Results ......................................................................................................................................35
       3.3.1. Phase 1/ Acquisition ...........................................................................................................36
       3.3.2. Phase 2/ Extinction ............................................................................................................36
       3.3.3. Phase 3/ Retrieval ...............................................................................................................36
       3.3.4. Difference in responding between context one and two .................................................37
       3.3.5. Control stimuli ...................................................................................................................38
   3.4. Discussion ..................................................................................................................................39
4. Dynamic coding patterns in single units of the avian forebrain across three stages of learning – a special role for prefrontal areas in extinction..................................................................................41
   4.1. Introduction ..............................................................................................................................41
       4.1.1. Single unit recordings during learning .................................................................................42
       4.1.2. The prefrontal-amygdala-hippocampal network .................................................................44
       4.1.3. The prefrontal cortex in learning .........................................................................................46
       4.1.4. Rationale and hypotheses ..................................................................................................47
   4.2. Materials and Methods ............................................................................................................48
I. List of figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Behavioral and neuronal mechanisms of (extinction-) learning and renewal.</td>
<td>5</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Schematic drawing of the pigeon brain.</td>
<td>13</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Overview of the paradigm of study one.</td>
<td>19</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Mean responses across acquisition, extinction and retrieval.</td>
<td>24</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Magnitude of context-specificity</td>
<td>26</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Context-independent responding to control stimuli.</td>
<td>27</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Test for latent inhibition.</td>
<td>28</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Mean amount of responding to stimuli across acquisition, extinction and retrieval.</td>
<td>37</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Context-specificity for acquired and extinguished responses</td>
<td>38</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Context-independent responding to control stimuli</td>
<td>38</td>
</tr>
<tr>
<td>Figure 11</td>
<td>Behavioral task of study three.</td>
<td>49</td>
</tr>
<tr>
<td>Figure 12</td>
<td>Behavioral Analyses</td>
<td>56</td>
</tr>
<tr>
<td>Figure 13</td>
<td>Exclusion criteria for recorded units and measurable effects of methodological improvement</td>
<td>57</td>
</tr>
<tr>
<td>Figure 14</td>
<td>Waveform clustering</td>
<td>59</td>
</tr>
<tr>
<td>Figure 15</td>
<td>Peri-Peck-Histogram for an example unit with motor-modulated activity.</td>
<td>60</td>
</tr>
<tr>
<td>Figure 16</td>
<td>Baseline and stimulus-modulated activity of four example units</td>
<td>61</td>
</tr>
<tr>
<td>Figure 17</td>
<td>Percentage of neurons differentiating between novel stimuli in different learning stages</td>
<td>63</td>
</tr>
<tr>
<td>Figure 18</td>
<td>Neuro-Behavioral Correlation</td>
<td>66</td>
</tr>
<tr>
<td>Figure 19</td>
<td>Example “acquisition remaining neuron”</td>
<td>68</td>
</tr>
<tr>
<td>Figure 20</td>
<td>Example “forgetting neuron”</td>
<td>69</td>
</tr>
<tr>
<td>Figure 21</td>
<td>Example “extinction neuron”</td>
<td>69</td>
</tr>
<tr>
<td>Figure 22</td>
<td>Example “behavior tracking neuron”</td>
<td>70</td>
</tr>
<tr>
<td>Figure 23</td>
<td>Unit classification</td>
<td>70</td>
</tr>
<tr>
<td>Figure 24</td>
<td>Correlation of activity of simultaneously recorded neurons</td>
<td>71</td>
</tr>
<tr>
<td>Figure 25</td>
<td>Selectivity Index (SI) across the learning stages</td>
<td>73</td>
</tr>
<tr>
<td>Figure 26</td>
<td>Histological results</td>
<td>73</td>
</tr>
</tbody>
</table>
II. List of tables

Table 1: Magnitude of context-specificity: ...............................................................26
Table 2: Overview of the paradigm in study two. ...............................................34
Table 3: Overview of reinforcement conditions and criteria in the three
        learning stages............................................................................................50
Table 4: Absolute number of neurons whose activity correlated with
        behavior. ........................................................................................................65
III. Abstract

Learning from positive and negative consequences displays a basic skill, which is shared by various organisms on this planet. In addition, to learn that some events in the environment can predict other critical ones is as important for survival as it is to learn that no relationship between two events exists anymore. These two learning processes are described by the terms acquisition and extinction, respectively. While much research was performed to elucidate the behavioral and neuronal mechanism of extinction learning in fear conditioning paradigms, insights into the mechanisms in appetitive designs are very limited.

This theses aimed at broaden the knowledge about extinction learning in an appetitive domain and investigated the mechanisms on a behavioral and neuronal level.

In two behavioral studies, we tested context-specificity of extinction memory and compared it to acquisition memory. We could show that the context-specificity for the extinction memory is as pronounced as for acquisition if these learning processes take place under the same conditions.

In the third study, we recorded the activity of single units in the avian functional analogue of mammalian prefrontal cortex (Nidopallium Caudolaterale, NCL), while pigeons acquired, extinguished and reacquired an operant response. We found two subgroups of neurons: One closely tracked behavioral performance, i.e. increased, decreased, and increased activity across the three learning stages (“behavior tracking neurons”). The other showed elevated activity restricted to the extinction phase (“extinction neurons”). We interpret these changes in activity as teaching signals for selecting the correct response during acquisition and reacquisition or inhibiting previous responses during extinction.
1. Introduction

To learn which stimuli are predictive for food under what circumstances is crucial for survival. Similarly, learning that formerly predictive stimuli no longer indicate food is advantageous in non-stationary environments. The first learning process describes the acquisition of a conditioned response while the second refers to extinction. Many decades of research point to the fact that the extinction of a conditioned response (due to withholding of the unconditioned stimulus, US) entails new learning. Using experimental procedures testing for spontaneous recovery, rapid reacquisition, reinstatement or renewal, it could be shown that the putatively forgotten association is still functional and extinction involves a second inhibitory learning process (Quirk and Mueller, 2008; Bouton et al., 2006). However, these phenomena also point to the fact, that this second learning process appears to be distinct from the first and clinical applications of extinction like exposure therapy are challenged especially by the observed context-specificity (renewal) of extinction learning. Consequently, the cause of this context-specificity as well as the neuronal mechanisms of the learning processes are subjective of substantial research efforts.

In my thesis, I will expand on this issue with three studies aimed at: 1) Investigating the context-specificity of an acquired as well as an extinguished response and 2) identifying neuronal mechanisms underlying learning in different stages (acquisition, extinction, and reacquisition) on the single neuron level.

For introduction, I will first describe behavioral learning mechanisms under appetitive and aversive conditions as well as the impact of context-specificity of learning. In addition, I will give an overview of the neuronal mechanisms of learning and comment on differences and similarities of acquisition and extinction. Finally, I will introduce the pigeon as an animal model and discuss differences and similarities of the avian and mammalian brain.

1.1. Learning on a behavioral level

Detecting relationships between events in the environment, predicting positive and negative occurrences and adapting behavior are extremely basic skills which are shared by a huge variety of organisms on this planet; from mice to men, from lumpfish to leeches and from bees to birds. This change in behavior due to experience is what defines learning:
“Learning is an enduring change in the mechanisms of behavior involving specific stimuli and/or responses that results from prior experience with those similar stimuli and responses” (Domjan, 2003)

Learning a response can include that the response is conditional to the outcome (operant conditioning) or that the outcome occurs independent of the response (classical conditioning), can be driven by positive and negative outcomes (appetitive and aversive unconditioned stimuli) and can involve that an event predicts the occurrence of an outcome (acquisition) or precisely the omission of it (extinction). These conditions will be discussed in more detail in the next three paragraphs.

1.1.1. Classical and operant conditioning

Importantly, the terms classical and operant conditioning both describe experimental arrangements where learning takes place, but they do not describe the learning process as such. During classical conditioning, an initially non-predictive (neutral) stimulus like a tone or light is repeatedly presented with a meaningful other stimulus (unconditioned stimulus) like food or foot shocks. Due to the temporal relationship between the occurrences of the stimuli, the neutral stimulus becomes predictive for the unconditioned one. As the organism learns that the stimulus is predictive for the unconditioned event, it starts to react to the stimulus in a way it normally responds to the unconditioned stimulus (unconditioned response). In a classical example, a dog starts to salivate to a tone, because it was repeatedly presented before he got fed. It was Ivan Pavlov who described this phenomena (Pavlov, 1927), accordingly this procedure is also called Pavlovian conditioning.

While in classical conditioning only the preexisting behavioral repertoire is exploited for the study of learning, operant conditioning entails the establishment of responses which the organism does not show spontaneously. During this procedure, a certain response becomes mandatory for an outcome to occur. This type of procedure is also called instrumental conditioning because an action of the organism is required for the outcome to happen.

In the first two studies reported in this thesis, animals undergo a classical conditioning paradigm (sign tracking) where the outcome of the trials does not depend on the subjects’ response; the third study involves operant conditioning: animals
learn to emit either of two different choice responses contingent to specific visual stimuli in order to obtain a food reward.

1.1.2. Acquisition and extinction learning

Above, learning was roughly defined as change in behavior due to experience. A change in behavior can be twofold: more or less responding compared to a previous time point. Therefore, learning can be associated with an increase as well as a decrease in conditioned responding. If a behavior is more often shown in response to a stimulus because it is followed by reinforcement, one speaks of acquisition. A decreased rate of responding after acquisition resulting from omission of reinforcement describes extinction. Even though a decreased response rate in general is often associated with forgetting, less responding due to withholding of the unconditioned stimulus cannot be interpreted as forgetting. Decades of research under various conditions (classical and operant conditioning employing both appetitive and aversive US) show that a once learned association is still functional after extinction of the response and is expressed also on the behavioral level under certain circumstances. A resurgence of responding after extinction can be observed when some time has passed by (spontaneous recovery), when the unconditioned stimuli was presented to the organism (reinstatement), when context cues from extinction were changed (renewal) or when reinforcement is re-introduced, leading to a more rapid increase in responding than during original acquisition (savings; rapid reacquisition). See figure 1A for an illustration of the effects. In the first two studies of this thesis, properties of extinction learning were investigated using a renewal procedure, in the third study using rapid reacquisition.

One might wonder, why the term extinction is then used for the decrease in response rate when the memory is still functional, since “extinction” implies the complete erasure of the learned association. Todd et al. (2014) describe this as the performance-memory-discrepancy where the response (performance) is extinguished but the memory still exists. Thus, the term extinction describes the phenomenon on the behavioral level, where no signs of the memory are expressed. However, the original memory persists and can be expressed under circumstances described above. The conclusion that the original memory is not abolished comes along with the assumption that during extinction a second association is built. The nature of this association is inhibitory and displays an alternative relationship between the CS and
US, namely that the CS does not longer predict the US (Bouton and Moody, 2004). In figure 1A, the discrepancy between the amount of behavioral responding and the strength of the association is illustrated. While during acquisition, responses on the behavioral level (black solid line) are increasing in parallel with associative strength (dotted black line), the curves diverge during extinction due to a decrease in response strength. In extinction, a second inhibitory connection (orange dotted line) is built which leads to the described decrease on the behavioral level. Finally, during retrieval, two connections exist in parallel and response strength can suddenly increase (black bar) when the first connection is expressed; for instance, when context cues change, renewal can be observed (see 1.1.4).

1.1.3. Appetitive and aversive unconditioned stimuli

Most research investigating the behavioral and neuronal mechanisms of extinction learning and renewal make use of classical auditory fear conditioning paradigms in rodents, i.e. an auditory cue (tone) is repeatedly presented before an electrical foot shock is applied (Herry et al., 2010). The conditioned response in this context represents the amount of freezing animals show, when the tone appears and is used as the dependent variable. In extinction, the tone is then presented without the foot shock and amount of freezing is decreasing. The reason for the extensive use of this experimental procedure to investigate extinction and context-specificity of learning is most likely twofold: first, this paradigm is widely accepted as an animal model of anxiety disorders (specific phobias). Thereby, extinction learning mimics the application of exposure therapy in a clinical setting. Second, fear conditioning is a straightforward experimental design in which animals learn and extinguish responses relatively fast – usually, within a few tens of trials on two or three days. Therefore, temporal, personnel and in the end financial effort is quite limited. However, most of human and animal behavior is maintained by positive reinforcement (reward), but we know much less about how organisms acquire and extinguish responses, which are reinforced by appetitive stimuli (US). In line with this, a recent series of experiments by Todd and Bouton investigated the extinction of operant conditioned responses with appetitive stimuli (Bouton et al., 2014). The authors conclude that the general mechanisms underlying the behavioral phenomena are similar to those during classical fear conditioning. As in those studies, the goal of
this thesis was to broaden the knowledge of behavioral and neuronal mechanisms underlying adaptive behavior maintained by positive reinforcement.

1.1.4. Context-specificity of learning

One argument that the first learned association is still functional even after the extinction of the response, arises from the renewal-effect which is defined as: “Recovery of learned performance after contextual cues that were present during extinction are changed” (Bouton et al., 2006). This influence of contextual cues is investigated using so-called ABA, AAB and ABC renewal designs, where the letters represent the respective context in which acquisition, extinction and testing for renewal takes place. A common finding in rodent behavioral studies is that these different designs are not equally potent to produce a renewal effect. The ABA design leads to a strong renewal effect while in AAB and ABC procedures the recovery of performance is much less pronounced (Bouton et al., 2006). Similar results were obtained when using an appetitive conditioning task in pigeons (Rescorla, 2008). The observation of AAB and ABC renewal led to the conclusion, that extinction memory is more context-dependent than acquisition, because acquisition transfers better to a new context (B or C). Besides that, it could be shown that a context switch following acquisition did not affect conditioned responding (Bouton and King, 1983), i.e. was not context dependent. However, there exist demonstrations of context-dependency of initial acquisition (Hall and Honey,
1990). This discrepancy of findings led to several theoretical considerations as well as empirical investigations, which will be taken up in the introduction of study one.

To conclude, the behavioral mechanisms involved in acquiring and extinguishing a response under appetitive and aversive conditions appear very similar and presumed differences probably result from different frequencies concerning the application of the paradigms. However, to draw a final conclusion more research in the appetitive domain is needed.

1.2. Neuronal mechanisms of learning

The adaptive value of learning lies in the ability to predict which events in the environment occur in close temporal relation to other salient events. This can be important for almost all behaviors which ensure survival or reproduction such as foraging for food, fleeing from predators or selecting mating partners. Likewise a huge network of regions encompassing almost the entire brain was found to be involved in the process of learning like the midbrain dopaminergic system, the hippocampus and amygdala as well as the prefrontal cortex (Salzman et al., 2005). Figure 1B depicts the mentioned brain regions and their interaction pattern. Amygdala, prefrontal cortex (PFC) and hippocampus are reciprocally connected (black arrows) and receive dopaminergic input from the VTA (blue arrows). Details concerning the respective function of the brain regions will be described in the next section.

1.2.1. Acquisition vs. extinction and appetitive vs. aversive outcomes – distinct or similar mechanisms?

Like investigations of learning on the behavioral level, one can divide the field by the question which learning process is investigated (acquisition vs. extinction) and if learning is driven by positive or negative consequences. However, again similar to the behavioral mechanisms, the brain regions involved in acquiring a response appear to be highly comparable to those involved in extinguishing a response. The same appears true for learning from positive and negative outcomes. An exception constitutes probably the prefrontal cortex which was shown to play a major role in extinction (Peters et al., 2009) by interacting with and executing top-down control over the amygdala and hippocampus. However, this is true for the aversive and appetitive domain. This topic will be taken up in the introduction of study three.
If one compares the brain regions assumed to be involved during acquiring or extinguishing a conditioned response in appetitive or aversive tasks, almost all studies mention the dopaminergic system, the amygdala, the hippocampus and the prefrontal cortex. From a systems perspective, this makes perfectly sense: extinction learning is assumed to present an inhibitory association which inhibits the association built during acquisition. To realize this inhibition, the same brain regions/connections should undergo plasticity during acquisition and extinction learning. Additionally, that a stimulus predicts or no longer predicts a positive or negative event does not occur in isolation but is often accompanied by other learning processes. For example, when one stimulus loses predictive power for an event (for example a food shock) in extinction, another could gain predictive power (for safety for example). Thus, the involvement of similar brain regions for these processes is to be expected.

Actually, one line of evidence concerning the argumentation that extinction involves new learning stems on the similar neuronal mechanisms of acquisition and extinction. For instance, extinction learning is dependent on the prefrontal cortex (Maren, 2013) modulated by brain derived neurotrophic factors (BDNF, Rosas-Vidal et al., 2014), involves neurogenesis (Akers et al., 2014) and requires protein synthesis like first acquisition (Nader et al., 2000; Nader and Hardt, 2009; Santini et al., 2004). Because of these active processes involved in the reduction of response rates, a passive forgetting mechanism cannot account for the behavioral changes seen during extinction.

A quite clear differentiation concerning the underlying brain mechanisms was classically made for valence defined as the degree of attraction or aversion. While the amygdala was mainly investigated in terms of regulating fear learning (Herry and Johansen, 2014; Maren and Quirk, 2004), dopaminergic signals in the ventral tegmental area (VTA) and the nucleus accumbens were regarded as a key player in appetitive learning (Schultz, 2013). However, more recent studies demonstrate a simultaneous involvement of brain structures for both extremes of the valence continuum. For the amygdala it was clearly pointed out, that it is equally involved in the processing of positive as well as negative events (Belova et al., 2008; Morrison and Salzmann, 2010; Morrison and Salzmann, 2009; Paton et al., 2006; Janak and Tye, 2015) The same is true for the VTA (Cohen et al., 2012; Lammel et al., 2014)
and nucleus accumbens (Carlezon, Jr. and Thomas, 2009), the prefrontal cortex (Peters et al., 2009) and the hippocampus (Haubrich et al., 2015). In addition, there are some hints that even if the same brain regions are involved, different subregions or different neuronal populations act on learning from appetitive and aversive consequences (Cohen et al., 2012). However, to draw a final conclusion about the equality of systems involved, more research in the appetitive field is needed. At this point the third study of this thesis will make an important contribution as it investigated the neuronal correlates of acquisition and extinction simultaneously in an appetitive design.

1.2.2. A neuronal network for adapting behavior to the environment

Above it was argued that similar brain networks participate in the adaptation of the organism to the environment independent of the direction of the behavioral change and the valence of the consequences. Therefore, I will describe the participating network as a whole and only point out differences where indicated.

What kind of information has to be processed in the brain to optimally predict important events and adapt behavior accordingly, i.e. to learn?

At the beginning, an organism should be able to define what is important or salient and if this important event is good or bad (rewarding or punishing). Neuronal signals reflecting the rewarding properties of an event are assumed to be implemented in the midbrain (ventral tegmental area, nucleus accumbens) receiving input from dopaminergic neurons in the substantia nigra (Schultz, 2013). Whereas aversive events have been shown to be processed in the amygdala and insula (Shin and Liberzon, 2010). As learning is defined by a change in behavior in relation to changes in the environment, learning only takes place when conditions in the environment change and the organism has to adapt to perform optimally. When the situation is as predicted, no learning takes place (Rescorla and Wagner, 1972). In the case of first acquisition a positive or negative event is not yet predicted by any stimulus in the environment, thus its occurrence is surprising or not expected. For extinction learning the non-occurrence of a predicted stimulus violates the expectations, again marking a highly salient event. Dopaminergic midbrain neurons are assumed to provide signals that encode the difference of what is actually received and what was expected (Schultz, 1998), i.e. changes in the environment. Studies
could show that these signals are indeed related to learning (Wise et al., 1996) and even causally involved in this process (Steinberg et al., 2013). Probably, the dopaminergic neurons receive information about aversive events (worse than expected or punishing) from the lateral habenula (Matsumoto and Hikosaka, 2009; Matsumoto and Hikosaka, 2007). In addition, dopaminergic neurons do not only project to the ventral midbrain but also to frontal cortices (Gaspar et al., 1992) where value (Padoa-Schioppa and Assad, 2006; Leon and Shadlen, 1999) as well as error signals (Carter and van, V, 2007) are processed. Value- or salience-associated signals have also been found in the amygdala (Janak and Tye, 2015) which is assumed to link stimuli in the environment and represent learned associations (Rogan et al., 1997). The storage of learned associations is also considered to be the function of the hippocampus. However, the hippocampus seems to combine the coding of different types of information important for associative learning, such as stimulus identity (Quiroga et al., 2005), time (Eichenbaum, 2014; Howard and Eichenbaum, 2014), context (Ji and Maren, 2007; Jin and Maren, 2015; Maren et al., 2013), outcome (Wirth et al., 2009) as well as spatial information (Moser et al., 2015). Finally, signals about the temporal linkage of events, their value and discrepancy between expectations and reality converges within the prefrontal cortex. There the appropriate action in the given situation (context) is selected and transmitted partly with the amygdala as a relay (Sotres-Bayon and Quirk, 2010) to motor output structures. In line with the assumed role of the PFC as a superior instance with executive control (Miller and Cohen, 2001), the PFC was repeatedly shown to be involved especially during extinction learning or when a situation is ambiguous (Sharpe and Killcross, 2014; Sharpe and Killcross, 2015; Rich and Shapiro, 2009; Rich and Shapiro, 2007). This displays one exception of the assumed equality of systems involved during acquisition and extinction. This topic will be further discussed in the introduction to chapter three as well as during the respective discussion part.

In summary, a neuronal network of different brain regions with the ventral midbrain, amygdala, hippocampus and prefrontal cortex as key structures participates in learning and the production of adapted behavior. Some critical information seems to be represented in different brain structures in parallel while the prefrontal cortex seems to have an outstanding role when situations become ambiguous during extinction.
1.3. Comparative considerations

The previous section described that learning constitutes a basic and critical skill for all organisms. Similarly, the behavioral and neuronal mechanisms underlying learning appear to be highly conserved across species (Mery, 2013). The marine snail *Aplysia* is the classical animal model for the investigation of neuronal mechanisms of classical (Carew et al., 1981) and operant conditioning (Brembs et al., 2002). And also other non-mammalian species are widely used to investigate the neuronal substrate of learning: for example bees (Eisenhardt, 2014); fruit flies (Burns et al., 2011; Mery, 2007) or *C. elegans* (Ardiel and Rankin, 2010); And all of these studies conclude, that the principal mechanism of learning on a neuronal level is evolutionarily highly conserved.

1.3.1. Birds in neuroscientific research

Birds are a common experimental animal in neuroscientific research: crows serve as a model for a variety of fields but mainly for comparative studies on avian and mammalian cognitive capacities and the underlying neuronal mechanisms (Moll and Nieder, 2014; Veit et al., 2014; Veit and Nieder, 2013); songbirds’ ability to learn a specific song during adolescence is taken as a model for speech acquisition (Brainard and Doupe, 2002). While the pigeon itself is a classical model in experimental psychology, it is also increasingly used as experimental animal in neuroscience research (Güntürkün et al., 2014; Colombo and Scarf, 2012). Most characteristics of a pigeon are extremely beneficial when performing research. Housing and feeding conditions are comparably inexpensive and handling of the animals is straightforward. However, they possess a multitude of cognitive capacities just as mammals (Güntürkün, 2005b). In experimental settings, they are easily motivated by food rewards. This results in an incredible endurance in behavioral experiments, as they are willing to perform several hundred trials in a row with a minimal amount of rewards. Finally, they are able to flexibly adapt to changing reinforcement conditions (Lengersdorf et al., 2014a; Starosta et al., 2013; Stüttgen et al., 2013; Stüttgen et al., 2011). Particularly the last study presented here made extensive use of these characteristics and approached the limit of what can be expected from experimental animals. In this specific paradigm (to be described and employed in
study three), animals were rewarded with probabilities between 60 to 85% and performed up to 1500 trials in a row while reinforcement conditions changed continuously. In this, they are on par with non-human primates.

1.3.2. Avian and mammalian brains

One of the most intensely studied regions of the avian brain in relation to cognitive functions is the Nidopallium caudolaterale (NCL). The NCL is argued to be a functional equivalent of the mammalian prefrontal cortex (Güntürkün, 2005a; Güntürkün, 2012; Kirsch et al., 2008) based on anatomical and functional studies: Like the mammalian PFC, the NCL is strongly innervated by dopaminergic fibers (Wynne and Güntürkün, 1995) and forms reciprocal connections with secondary and tertiary sensory fields of all modalities (Leutgeb et al., 1996). Additionally - and of enormous importance when investigating the neuronal substrate of (extinction) learning - the interconnections of the NCL and amygdala resemble the connections seen between the mammalian PFC and amygdala (Kröner et al., 2002). In figure 2 the reciprocal connections of the NCL (red) with all sensory fields, efferent connections to motor-output structures and the input from thalamus and dopaminergic regions are depicted. On a functional level, it could be shown that the NCL is involved in working memory (Diekamp et al., 2002a; Diekamp et al., 2002b; Rose and Colombo, 2005), cognitive flexibility (Diekamp et al., 2000; Hartmann and Güntürkün, 1998; Lissek et al., 2002), decision making (Kalenscher et al., 2005; Kalenscher et al., 2003), reward processing (Kalenscher et al., 2005; Kalenscher et al., 2006), (extinction-) learning (Diekamp et al., 2000; Kirsch et al., 2009; Kirsch and Güntürkün, 2005; Lengersdorf et al., 2014b; Lissek and Güntürkün, 2003; Lissek et al., 2002) and attention (Rose et al., 2010). All of these characteristics are also associated with mammalian prefrontal cortex function (Miller and Cohen, 2001).

The first studies conducting single unit recordings in the NCL investigated the functional properties of NCL neurons during a delayed Go/ NoGo task in a head-fixed preparation (Diekamp et al., 2002b; Kalt et al., 1999). Both studies showed elevated activity during a delay period of the task (between stimulus presentation and outcome delivery), which was interpreted as a working memory signal since it was modulated by the meaning or relevance of the previously presented stimulus. In
contrast, the majority of studies investigated the processing of reward-related information. In a number of experiments, the activity of NCL neurons was recorded during the delay phase of a trial again. The authors found elevated activity during this phase which was interpreted as a working memory signal (Rose and Colombo, 2005), since it was only present if remembering was necessary. However, follow-up studies (Browning et al., 2011; Milmine et al., 2008) could show that the elevated firing rate was rather associated with the probability of an upcoming reward than the representation of stimulus identity. Finally, single units in the NCL were shown to differentiate between different amounts of reward at reward delivery and during presentation of reward predicting cues (Koenen et al., 2013) as well as to integrate reward amount and time-to-reward (Kalenscher et al., 2005; Kalenscher et al., 2006). Starosta et al. (2013) investigated the responsiveness of NCL neurons in a generalization task where grating patterns were used as visual stimuli, differing in spatial frequency and similarity to previously trained stimuli (S+ and S-). They found that neurons in the NCL increased their firing in response to stimuli similar to the S- as well as when an expected reward was omitted. In addition coding of sensorimotor characteristics of the task was found, leading to the interpretation that the (negative) value of stimuli, the associated response as well as the outcome is coded in the NCL. In a very recent study conducting single unit recordings in NCL, animals were performing a visual categorization task under perceptual uncertainty (Lengersdorf et al., 2014a). Here, a subset of neurons was coding for stimulus identity. Their activity level during stimulus presentation was ramping to the end of the sample presentation epoch and predictive for the subsequent choice response. This resembles activity patterns seen in monkey intraparietal and prefrontal area that were interpreted as signals for accumulated sensory evidence during decision-making (Kim and Shadlen, 1999; Shadlen and Newsome, 2001). Summarizing the described results of electrophysiological recordings in the NCL, one can state that the response pattern closely resemble those seen in mammalian PFC.

In conclusion: The NCL is a multimodal forebrain structure involved in the translation of sensory information into goal-directed actions and in this respect closely resembles the mammalian PFC. Additionally, functional coding properties of single units within the NCL are similar to those seen in mammalian PFC. Furthermore, pigeons are especially well suited to investigate neuronal mechanisms of learning due to their behavioral characteristics. Finally, because of the implementation of
similar neuronal mechanisms underlying learning across species, the insights about neuronal mechanism of learning in the pigeon brain can aid in the search for general principles of brain function and thus shed light on learning in other vertebrate species, including humans.

Figure 2: Schematic drawing of the pigeon brain. The reciprocal connections of the Nidopallium Caudolaterale (NCL) with secondary and tertiary sensory fields (yellow/orange), to motor-related output-structures (green) and the amygdala (brown) are shown. In addition, inputs from the nucleus dorsolateralis posterior thalami (DLP) as well as from the ventral tegmental area (AVT) and substantia nigra (SN) are depicted. Modified after Güntürkün, 2005.
2. **Influence of expectancy violation on context-specificity of acquisition and extinction in a appetitive sign-tracking task**

2.1. **Introduction**

A general assumption concerning the context-specificity of different learning processes is that extinction is more context-specific than acquisition (see Rosas et al., 2013 for a recent review). This derives mainly from two experimental observations: 1) perfect transfer of acquisition performance between contexts and 2) AAB and ABC renewal. The first mentioned observation that acquisition memory generalizes to a new context, was observed in a classical experiment using an ABA renewal paradigm (Bouton and King, 1983). Rats were trained in a fear conditioning paradigm to associate a tone with a food shock in one context and were subjected to a second context for extinction training. Since the authors observed no drop in performance when the context was changed as well as identical rates of extinction, they concluded, that acquisition was not context-dependent. The fact that AAB and ABC procedures also lead to a renewal effect as the classical ABA design (Bouton and Bolles, 1979; Laborda et al., 2011; Polack et al., 2013) implies that extinction is more context-specific than acquisition, because original conditioning transfers better to the new context (Bouton, 2004).

The pronounced context-specificity of extinction learning challenges clinical applications of this form of learning as for example behavioral therapy of phobias. Consequently, reasons for the remarkable context-specificity of the extinction memory were highly discussed and led to various theoretical considerations. For example, Bouton, (2004) claimed that during extinction a second inhibitory association is built and that the context-specificity of extinction origins in the circumstance that it represents a second alternative association regarding the stimulus. He assumes that context is only incorporated into the memory trace if it helps to successfully act on a task. During acquisition, no contextual information is necessary to do so, in turn less attention is directed to the context and in the end acquisition is less context-specific. Experiments investigating the orienting responses during learning observed a decline in attention to a CS during acquisition and a recovery during extinction (Kaye and Pearce, 1984). It was argued that contextual stimuli during acquisition are from some point on no longer processed until the surprise engendered by the omission of the US at the outset of extinction encourages the organisms
to pay attention to the actual context. The assumption that the expectancy violation experienced during extinction triggers attentional processes was supported by behavioral and neurophysiological experiments (Vachon et al., 2012; Dickter and Gyurovski, 2012). A critical factor for Bouton as well as Pearce (Darby and Pearce, 1995) displays the ambiguity associated with a stimulus when it is no longer followed by the US. Then, the context is used by the organism to deal with this ambiguity. Consequently, only from the extinction phase on context is important and will be processed, leading to a stronger context-specificity for extinction.

However, when a new stimulus is introduced and followed by a reward or aversive stimulus, this can also be regarded as surprising or unexpected and boosting attention to the context. In line with this argumentation, there is evidence for context-specific acquisition memory. (Lucke et al., 2013; Hall and Honey, 1990; Rosas and Callejas-Aguilera, 2006; Harris et al., 2000). More recently, Bouton (Bouton and Todd, 2014) discussed a series of experiments where context dependent storage of (non)-extinguished operant behavior is shown. He concludes, that there is probably a difference between instrumental and classical conditioned behavior with regard to the context-specificity of acquisition, because a context switch from acquisition to extinction, decreased performance. However, also for operant behavior he argues that extinction memory is more context-specific than acquisition, because of the demonstrated ABC and AAB renewal-effect.

Similar to the ideas of Bouton and Pearce concerning ambiguity, Rosa and others (Rosas et al., 2006) proposed the attentional theory of context processing (ATCP), where ambiguity and subsequent attention to the context induced by extinction plays a major role. He claimed that associations are stored in a context-specific way as soon as ambiguity is experienced regardless of the nature of the association (inhibitory vs. excitatory; first vs. second learned; acquisition vs. extinction), because of the attention paid to the context. Thereby, it is not important that the ambiguity was experienced for a specific stimulus. A recent experiment in rats is supporting the theory (Bernal-Gamboa et al., 2014). They observed that an association becomes context-specific once individuals experienced ambiguity even though in a different task.

In addition, the experienced relevance of a context is assumed to be important when one investigates context-depending learning. A recent experiment investigated the
influence of context relevance as well attention to the context (Lucke et al., 2013).
Two groups of participants received discrimination training where the context was
either relevant or irrelevant. It was found, that the renewal effect was more pro-
nounced in the group which learned that the context is relevant. In addition, fixation
times for the respective stimuli revealed longer fixation and thus more attention
directed to the relevant stimuli. Similar effects were observed for extinction
memory (Lucke et al., 2014).

In conclusion, experimental data until now supports the view that context-specific-
ity of acquisition and extinction do not differ because of a fundamental difference
in the learning processes. Rather, acquisition is less context-specific, because no
ambiguity is experienced and no attention is paid to the context.

However, there exists some experimental evidence, that under appetitive conditions
the ambiguity experienced for one stimulus does not necessarily lead to context-
specificity of performance for another one (Nelson et al., 2011). This challenges the
proposed mechanisms and questions the general validity of the previously described
theoretical assumptions and experimental results. However, the main theoretical as-
sumption is that acquisition and extinction underlie the same learning rules and dif-
fences in the context-specificity occur, because of the circumstances during ex-
tinction as for instance the stronger expectancy violation. This proposal could be
tested if acquisition and extinction take place simultaneously, i.e. under exactly the
same circumstances. Then, context-specificity should be equal for acquisition and
extinction.

To investigate under appetitive conditions if indeed the circumstances under which
memory develops are critical for the context-specificity of memory, we tested the
context-specificity of acquisition and extinction memory in the same situation, i.e.
simultaneously regarding different stimuli. In addition, we were interested if the
amount of expectancy violation and subsequently attention paid to the context in-
fluences the extent of context-specificity.

For this purpose, we designed a task, where one group of animals experienced a big
expectancy violation and the other a small one while both were acquiring and ex-
tinguishing a conditioned response simultaneously. This allows us to investigate the
influence of expectancy violation on extinction and acquisition in parallel. At the
end of the experiment, we tested animals for latent inhibition by subjecting them to an autoshaping procedure with a cue presented during the extinction phase. Latent inhibition has been defined as “decrement in learning performance which results from the nonreinforced preexposure of the to-be-conditioned stimulus” (Lubow, 1973). It was argued that the nonreinforced preexposure to a stimulus leads to a decrement in attention towards it. Thus, a stronger preexposure effect implies more attention to the stimulus during preexposure. And if we see latent inhibition to a different extent between groups, this would be a marker of more attention paid to the context.

In detail, we expect the group experiencing a big expectancy violation (BEV) to show more context-specific responding in comparison to the group experiencing a small expectancy violation (SEV), because they directed more attention to the context in the extinction phase. This should be true regardless of whether the response was recently acquired or extinguished. Based on the results of Rosas (Bernal-Gamboa et al., 2013; Rosas et al., 2013; Rosas and Callejas-Aguilera, 2007), we predict that acquired and extinguished responses are context-specific to a similar degree. If attentional processes determine how context-specific a memory is formed, latent inhibition should be more pronounced in the BEV group, because animals in this group directed more attention to the context and learned that the cue (later used for autoshaping and test for latent inhibition) in the extinction context did not predict any food.

2.2. Materials and Methods

2.2.1. Subjects

Twenty-five experimentally naive homing pigeons (Columba livia), obtained from local breeders and raised in the institute’s own aviary, served as subjects. Animals were housed in groups of eight animals in an aviary with a 12 h dark-light cycle (lights off at 8 p.m.). Water was available at all times; food was restricted to the period of daily testing on workdays, with additional free food available on weekends. During the experiment, the pigeons were maintained at 85–90% of their free-feeding weight. All subjects were treated according to the German guidelines for the care and use of animals in science. All procedures were approved by a national ethics committee of the State of North Rhine-Westphalia, Germany.
2.2.2. Behavioral apparatus

Two operant chambers were used in this experiment, each measuring 34cmx34cmx50cm. The back wall of each chamber featured two translucent response keys (4 cm by 4 cm, bottom height from the floor 17 cm) which could be transilluminated by an LCD flat screen mounted against the back wall of the experimental chamber. Each effective key peck produced an audible feedback click and was registered. Food (grain) was provided by a food hopper located below the center key. Each chamber was housed in a sound-attenuating shell. In one chamber (physical context one) the walls were lined with yellow wallpaper and loudspeakers provided brown noise, whereas in the other chamber (physical context two), the walls were painted red and white noise was audible. Please note that the physical contexts were labeled as context one and context two whereas the classical terminology of an ABA renewal designs will be used within the experiment (see below). All hardware was controlled by custom-written MATLAB code (The Mathworks, Natick, MA) using the Biopsychology Toolbox (Rose et al., 2008).

2.2.3. Procedure

In general, the experiment consisted of a sign-tracking procedure, meaning that the US presentation was not dependent on the animals’ response. There were three training/testing phases, which took place in different physical contexts (A, B, A/B-renewal design). In addition, there was a pretraining phase in which animals were habituated to the contexts and confronted with two control stimuli (one CS+ (Target); one CS– (nonTarget)). The stimulus set included twelve colored patterns of which ten were randomly assigned to each animal. See figure 3A for a depiction of the sequence of events within one trial.

2.2.3.1. Pretraining

This phase lasted six weeks. The physical context alternated daily. Animals received one week training in an autoshaping procedure: one stimulus (Target) was presented at the center key for 5 s and followed by a reward (1.5 s access to food). The Inter-Trial-Interval (ITI) was 120 s. In the second week of training, a second stimulus (nonTarget) was introduced. Its presentation was not followed by food. Target and nonTarget presentation was alternating randomly within the session. In
the next five weeks of training, the ITI was stepwise reduced to 20 s while the number of trials was increased to 140 trials per session. Animals which did not show differential pecking to the Target- and nonTarget-stimulus at the end of this phase, did no longer participate in the experiment.

2.2.3.2. Phase 1/ Acquisition

In this phase, all animals received the same training. Half of them were trained in the physical context one, the other half in physical context two. To keep terminology consistent with classical ABA-Renewal designs, the context in which animals received training in phase one/ acquisition will be denoted as context A from now on, while the context in which extinction training in phase two took place will be denoted as context B even though contexts can be physically different (counterbalanced across animals). In addition to Target and nonTarget, five novel stimuli were introduced in phase one. Two stimuli (CS1+, CS3+) were followed by a small amount of food (1.5 s), one (CS2++) by a big amount of food (7 s) and two filler
stimuli (FS1-, FS2-) were not followed by food. See figure 3B for an overview of stimulus composition and reward contingencies across sessions and experimental groups. This phase consisted of 15 daily sessions (except weekends) with 20 trials per stimulus and two additional start-up-trials with Target presentation at the beginning of each session. Sample presentation time was 5 s and the ITI lasted 20 s. This added up to 142 trials per session/ 1 h training per day.

2.2.3.3. Phase 2/ Extinction

Extinction training took place in the respective other context than acquisition phase. Half of the animals were assigned to the big expectancy violation (BEV) group and the others to the small expectancy violation (SEV) group. In the BEV group, stimulus CS2++ was presented but no longer followed by food (CS2−) and stimulus CS3+ was no longer presented at all. In the SEV group, the stimulus composition concerning CS2++ and CS3+ was the other way around. This way, we aimed at manipulating the experienced expectation violation. Stimulus CS1+ was no longer followed by food in both groups. In addition, three novel stimuli were presented to all animals. Two of them were followed by a small amount of food (CS5+, CS6+) and one by a big amount (CS4++). These stimuli fulfilled two purposes: 1) keeping the amount of reinforcers equal between contexts and 2) serving as stimuli to which responding was established while responding to others was extinguished. During the whole session, a black and white (b/w) pattern was presented at another pecking key. This stimulus served as a CS+ in the test for latent inhibition later in the experiment. The extinction phase consisted of four daily sessions. Trial number, sample presentation time and ITI were the same as during acquisition.

2.2.3.4. Phase 3/ Retrieval

This phase consisted of two sessions (one in each in context) which took place at the same day, separated by one hour. If animals were tested in the respective extinction context (B), the b/w pattern was presented at the side key during the whole session. With exception of the Target stimulus, each stimulus was presented twelve times and tested in extinction. The Target stimulus was continuously followed by reward and presented as often as all stimuli together to maintain motivation of the animal. Each session consisted of 90 trials. Sequence of context tested was randomized across animals.
2.2.3.5. Test for latent inhibition

After the retrieval phase, animals were subjected to an autoshaping procedure for three consecutive sessions/ days in their respective extinction context. The b/w pattern was presented for 4 s at the side key and followed by food for 1.5 s The ITI was 20 s and every session consisted of 60 trials.

2.2.3.6. Analyses

The absolute amount of pecking in response to presented stimuli served as the dependent variable in this experiment. We performed one and two-way repeated measures of variance (ANOVA) and paired t-test. All analyses were conducted in MATLAB (The Mathworks, Natick, MA; statistical toolbox).

2.3. Results

If animals showed at any point of the experiment no sign of differentiation between rewarded and non-rewarded stimuli anymore, the data of these animals was not subjected to the final data set. This resulted in complete data sets for 13 animals (eight in the SEV group, five in the BEV group)

Figure 4 shows the mean responses to stimuli CS1, CS2/CS3, CS4, CS5, CS6 across animals for the SEV (upper panel, A) and BEV (lower panel, B) group in the three testing phases (acquisition, extinction, retrieval). Responses to filler cues (FS1, FS2) are not shown; responses to control stimuli were depicted in a separate figure (figure 6).

2.3.1. Phase 1/ Acquisition

During phase one/ acquisition three new stimuli (CS1, CS2, CS3) were introduced and their presentation was followed by food. The mean response rate to stimuli in blocks of ten trials is shown in the leftmost panels of figure 4. A two-way repeated measures ANOVA revealed for every stimulus a significant main effect for block (CS1: $F(4, 27) = 171.9, p < 0.001$; CS2: $F(4, 27) = 271.31, p < 0.001$; CS3: $F(4, 27) = 132.77, p < 0.001$) and group (CS1: $F(1, 4) = 7.32, p = 0.009$; CS2: $F(1, 4) = 24.63, p < 0.001$; CS3: $F(1, 4) = 11.68, p = 0.001$) as well as an interaction of block and group (CS1: $F(4, 1) = 209.67, p < 0.001$; CS2: $F(4, 1) = 310.78, p < 0.001$; CS3: $F(4, 1) = 155.41, p < 0.001$). This reflects an increasing response rate over the
time course of the acquisition phase (significant effect of block) for both groups and a higher response rate for group BEV (group effect) which was more pronounced at the beginning of the phase (interaction). Since the groups did not undergo any different procedure until this time point, difference in the mean response rate between them solely reflects differences of response tendency between single animals which are more pronounced in a group with a more restricted sample size (see discussion).

2.3.2. Phase 2/ Extinction

In phase two/ extinction animals were separated into two groups (BEV, SEV). While group SEV was confronted with stimuli CS1 and CS3, to group BEV stimulus CS1 and CS2 were presented. All three CS were no longer followed by food. Extinction training took place in a different context (B) than acquisition (A).

To test for the chances in responding due to withholding of the US for the CS1 CS2/CS3, we compared the mean amount of pecking across blocks of ten trials and between groups. For stimulus CS1 a two-way repeated measures ANOVA revealed a significant main effect of block \( (F(7, 4) = 59.32, p < 0.001) \), but not of group \( (F(1, 4) = 0.16, p = 0.696) \) and a significant interaction of block and group \( (F(4, 1) = 155.41, p < 0.001) \), reflecting a decreasing response rate over time for both groups with higher response rate in the BEV group at the beginning of the phase. Stimulus CS2 and CS3 were only presented in either of the groups and a one-way repeated measures ANOVA yielded for both stimuli a significant effect of block \( (CS2: F(4, 6) = 22.76, p < 0.001; CS3: F(7, 6) = 28.39, p < 0.001) \), meaning that for both stimuli responses were decreasing over time, i.e. responses extinguished.

To keep the amount of reinforcement constant for both contexts and to test for simultaneous acquisition of responding, we introduced three new stimuli in both groups. Their presentation was followed by food for 7 s (CS4) or 1.5 s (CS5, CS6).

A successful acquisition should be observable in increased responding over the time course of phase two. We compared the amount of pecking across blocks for specific stimuli between groups. For stimuli CS4 and CS6 the two-way repeated measures ANOVA yielded a significant main effect of block \( (CS4: F(4, 6) = 119.04, p < 0.001; CS6: F(4, 6) = 125.51, p < 0.001) \), but not of group \( (CS4: F(1, 6) = 1.49, p = 0.246; CS6: F(1, 6) = 0, p = 0.981) \) and a significant interaction of block and
group (CS4: $F(1, 4) = 25.33, p < 0.001$; CS6: $F(1, 4) = 55.15, p < 0.001$), reflecting an increasing response rate and more responses of the BEV group at the beginning of the phase. Results for stimulus CS5 were comparable concerning the effect of block ($F(4, 6) = 119.04, p < 0.001$) and the block x group interaction ($F(1, 4) = 122.46, p < 0.001$). In addition, a main effect of group could be observed ($F(1, 6) = 30.35, p < 0.001$), reflecting an increasing response rate for both groups over the time course of phase two as well as a higher response rate of group BEV in general and more pronounced at the beginning of the phase.

In summary, the results of phase one and two show increases in responses for stimuli which were followed by food while responses to stimuli which were no longer followed by food decreased. In addition, a general higher response rate for group BEV was observed what was probably due to the restricted sample size (see discussion). Overall, behavior in phase one and two built not the focus of the present study and was not investigated in more detail.

2.3.3. Phase 3/ Retrieval

In the retrieval phase, responding to CS3 (SEV) or CS2 (BEV) and CS1, CS4, CS5 and CS6 was tested in extinction in both contexts.

As described above, we expected context-specific responding for acquired as well as extinguished responses. For all stimuli, we expected more responding in their respective acquisition context. Thus, for stimuli CS2/CS3 and CS1 to which responding was extinguished recently in context B, we predicted more responding in context A than in context B. For stimuli CS4, CS5 and CS6 to which responding was recently acquired in context B, we predicted more responding in context B than in the A.

The rightmost panel of figure 4 shows the mean amount of pecking across twelve test trials in both contexts and both groups (upper (SEV) and lower (BEV) panel) and to stimuli to which responding was recently extinguished (blueish area) or acquired (reddish area). Dotted bars present the responses in the respective acquisition context. Left bars are associated with responding in the context A and right ones in context B.
The following is true for both groups: for stimuli CS2/CS3 and CS1 strong responding in the context A (left/lighter bars) was observable, which is widely known as the renewal-effect. Whereas, responding to CS4, CS5 and CS6 were more pronounced in the context B (right/darker bars). In summary, animals responded more to stimuli when they were presented in their respective acquisition context (dotted bars). The described result were confirmed by a two-way repeated measures ANOVA for stimuli CS1, CS4, CS5 and CS6 and a one-way repeated measures ANOVA for stimuli CS2/CS3: significant main effect of block (all $p < 0.001$) but not of group (all $p > 0.05$) and no interaction of block and group (all $p > 0.05$).

**Figure 4: Mean responses across acquisition, extinction and retrieval.** Mean amount of pecking in blocks of 10 trials split up for stimuli (color-code), phases (acquisition, extinction, retrieval) and groups (A, B). A) The leftmost panel shows responding to CS1 (cyan), CS2 (dark blue) and CS3 (light blue) during acquisition. In the central panel amount of pecking during extinction is shown for CS1, CS3 as well as CS5 (yellow), CS6 (orange) and CS6 (red). Mean responses in blocks of 12 trials during retrieval in both contexts are shown in the rightmost panel. Left bars represent responding in context A and right bars responding in context B. Dotted bars show responding in the respective acquisition context. Color codes were like before. Errorbars depict Standard Error of the Mean (SEM). B) Same as in A) but for group BEV and for CS2 instead of CS3 during extinction and retrieval.
2.3.4. Difference in responding between contexts

As described above, we were interested in the degree of context-specificity of the memory between groups as well as between acquired and extinguished responding to the specific stimuli. The difference in responding to the stimuli between contexts can be interpreted as a measure for the degree of context-specificity. Thus, we took the absolute difference between responding in context A and responding in context B for the five stimuli presented in the retrieval phase. This difference is shown in figure 5 for the two groups in the left (A, SEV) and right panel (B, BEV).

To test for possible differences between stimuli or groups, we computed a two-way repeated measure ANOVA with the factors stimuli and group. It yielded no significant effect of stimuli ($F(1, 4) = 0.77, p = 0.551$) or group ($F(4, 1) = 1.69, p = 0.220$) and no significant interaction ($F(1, 4) = 0.82, p = 0.517$), showing that context-specificity was comparably strong for different stimuli (to which responding was recently acquired or extinguished) and there was no effect of the manipulation of reward expectancy in the two groups.

Since visual inspection of the data points to a stronger effect in the BEV group and absence of evidence is not evidence of absence, we computed the effect sizes for the context effects for both groups and all stimuli. The results are shown in table 1. Effect sizes of both groups are a very heterogeneous (range 0.8-2.5) and effects within one group for different stimuli are larger than between groups. Between groups, average effect sizes are similar for all stimuli (mean all: 1.62 (SEV) vs. 1.73 (BEV)). The same is true for extinguished vs. acquired responses (SEV: 1.56 vs. 1.66, BEV: 1.88 vs., 1.62).

This means that the renewal-effect as well as context-specific responding to stimuli to which responding was recently acquired was comparably strong between groups. Furthermore, this can be interpreted in a way that context-specificity is comparably strong for acquired and extinguished responses.
Figure 5: Magnitude of context-specificity. Difference in responding. A) Data for group SEV. Absolute difference in responding between contexts in the retrieval phase split up for stimuli for which responses were recently acquired (red shaded area) or extinguished (blue shaded area). Bars represent different stimuli. Errorbars display Standard Error of the Mean (SEM). B) Same as in A, but for group BEV.

Table 1: Magnitude of context-specificity: Effect sizes (hedges g) for the difference in responding between contexts split up for stimuli, acquired and extinguished responses and groups are shown.

<table>
<thead>
<tr>
<th></th>
<th>SEV</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>BEV</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CS1+/−</td>
<td>CS3+/-</td>
<td>CS4++</td>
<td>CS5+</td>
<td></td>
<td>CS1+/-</td>
<td>CS2+/-</td>
<td>CS4++</td>
</tr>
<tr>
<td>effect size</td>
<td>1,36</td>
<td>1,76</td>
<td>2,51</td>
<td>1,37</td>
<td>2,01</td>
<td>effect size</td>
<td>2,00</td>
<td>1,56</td>
<td>0,87</td>
</tr>
<tr>
<td>mean Ac&amp;Ex</td>
<td>1,56</td>
<td></td>
<td>1,66</td>
<td></td>
<td></td>
<td>mean Ac&amp;Ex</td>
<td>1,88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean all</td>
<td>1,62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mean all</td>
<td>1,73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.3.5. Control stimuli

Two stimuli were presented throughout all phases of the experiment and were always (Target) or never (nonTarget) followed by food. To test for possible context-specific responding to these stimuli, we compared amount of responding also for these across contexts. However, we did not expect to observe context-specificity for these stimuli, because animals experienced context-irrelevance throughout the experiment for them, i.e. conditions were the same in both contexts for Target and
nonTarget. Figure 6 shows the average number of responses to Target and nonTarget stimuli in the two contexts and both groups (panel A and B). As expected, average amount of responding for control stimuli where comparable low (nonTarget) and high (Target) in both contexts and groups. This was confirmed by a two-way repeated measures ANOVA for Target and nonTarget stimuli which yielded neither a significant main effect of context (Target: $F(1, 4) = 3.11 \, p = 0.108$; nonTarget: $F(1, 4) = 1.75 \, p = 0.213$), nor of group (Target: $F(1, 4) = 0.57 \, p = 0.466$; nonTarget: $F(1, 4) = 0.22 \, p = 0.213$) nor a context x group interaction (Target: $F(1, 4) = 0.1 \, p = 0.764$; nonTarget: $F(1, 4) = 0 \, p = 0.989$).

![Figure 6: Context-independent responding to control stimuli. A) Mean responses to the Target (black) and nonTarget (grey) stimulus for both contexts. No context-specificity is observed. Errorbars show Standard Errors of the Mean (SEM).](image)

2.3.6. Latent inhibition

To assess the amount of attention paid to the context during extinction, we made use of a phenomenon called latent inhibition as described above.

To use this indirect measurement of attention, we paired a cue presented during extinction as part of the context with food afterwards. If animals paid more attention to the context (cue) during phase two of the experiment, it should be more difficult for them to learn that this cue is now predictive for food (latent inhibition). Less responding to the cue represents therefore an indicator for more attention paid to the context during phase two/ extinction.
Figure 7 shows mean responses to the cue during autoshaping across animals for the two groups. A two-sample t-test yielded no significant difference in responding between the groups ($t(11) = 1.25, p = 0.236$).

**Figure 7: Test for latent inhibition.** Mean amount of pecking split up for group SEV and BEV during the test for latent inhibition is shown. Errorbars show Standard Errors of the Mean (SEM).

### 2.4. Discussion

The aim of the present study was to investigate the context-specificity of responding which was recently acquired or extinguished and the influence of expectancy violation on the extent of context-specificity. We tested two groups of pigeons in a sign-tracking paradigm where they acquired and extinguished the same response (pecking) to different visual stimuli simultaneously in a context different from first acquisition. One group of animals experienced a small expectancy violation during learning (omission of a small reward) and another one a large violation (omission of a large reward). Whereas we could not find any influence of this manipulation on the extent of context-specificity, we observed strong context-specific responding for both recently extinguished as well as recently acquired responses.

#### 2.4.1. Comparison of acquired and extinguished responses

To compare context-specificity for acquired and extinguished responses, we let animals acquire a response to specific stimuli while simultaneously responding to other ones was extinguished. This learning took place in a different context than first acquisition. When tested in both contexts again, we saw for both acquired and extinguished responses elevated responding in the respective acquisition context (figure 4 rightmost panel, and dotted bars). This results contrasts with theoretical
considerations that claim no influence of context for the first association built because no ambiguity in regard to the predictive value of the stimulus is experienced (Bouton, 2004). In our task, the first association animals learn in regard to CS4, CS5 and CS6 is that they predict food and consequently the animals acquire responding to these stimuli in phase two (figure 4, central panels). Nevertheless, animals responded in a context-specific way in phase three to these stimuli (less responding in context A, left, solid bars). Moreover, the extent of context-specificity assessed by the effect sizes (see table 1) of the differences in responding between the contexts suggests that responding to the stimuli CS4, CS5 and CS6 is as context-specific as responding to the stimuli to which responding was extinguished (CS1, CS2/3). These results are perfectly predicted by the ATCP (Rosas et al., 2006) as one of the main assumptions is that not the kind of memory (first vs. second learned and excitatory vs. inhibitory) but the circumstances (if ambiguity is experienced in general and attention paid to the context) is crucial. Because in our task, acquisition and extinction took place under exactly the same circumstances (simultaneously) and we saw the same extent of context-specificity for acquisition and extinction, it strongly supports the theory.

One could argue that the observed context-specificity of acquisition results from facilitated extinction due to the context change from the second to the third phase. Because we tested in extinction during the third phase, less responding could be the explained by this. However, the testing consisted of 12 trials only, and the context change from phase one to phase two did not induce such a strong difference in responding within the first trials (compare end of acquisition to first block of extinction). This implies that a context change in general and facilitated extinction cannot account for the differences seen between the contexts in phase three.

Interestingly, we saw no context-specific responding for the control stimuli (figure 6), most likely because animals were taught during pretraining, that for these stimuli context is irrelevant (Lucke et al., 2013; Lucke et al., 2014). However during extinction, animals got to know, that for the two other stimuli (CS1 and CS2/CS3), the context is relevant (reward in context one, no reward in context two). That means that when animals acquire a response to newly introduced stimuli during phase two/extinction, their guess if context is relevant should be 50% (control stimuli no, new stimuli yes). However, experimental results for the acquired responses
imply, that animals generalize the context-relevance to the new stimuli, because they show context-specific responding in the retrieval phase. This could imply a mechanism for context-relevance in a way that if a new association is built it is susceptible for context-specific storage once context relevance was experienced. The explanation that animals apply the rule that they experience at the moment (context is relevant) does not hold in this scenario, since animals continuously experience context-irrelevance for the control stimuli, also.

In summary, we observed no context-specific responding as long as animals were taught that the context is irrelevant (control stimuli). However, once animals experienced context relevance (during phase two for CS1, CS2/CS3), associations built from this time point one were context-specific regardless of their kind.

2.4.2. Influence of expectancy violation

We aimed at manipulating the experienced expectancy violation by confronting different groups of animals with the omission of a reward of different size during extinction. We expected that this manipulation would lead to differences in context-specific responding mediated by the amount of attention paid to the context. Namely, we expected a stronger renewal effect as well as context-specific responding in the group which experienced a large expectancy violation (BEV) compared to the group which experienced a small expectancy violation. However, we could not find any difference in context-specific responding between the experimental groups.

The reasons for this are most likely manifold. First, it is possible, that the chosen manipulation of expectancy violation via the amount of reward omitted was not as salient for the animal as expected. We choose feeding times of 1.5 vs. 7.s as small and large reward. Already during acquisition we did not detect any signs of differentiation on the behavioral level between stimuli followed by a small or large amount of food even though a previous study did show an effect of reward size on conditioned responding or learning using more similar reward sizes (Rose et al., 2009). However, in this study percent of correct choices instead of amount of pecking was used as dependent variable. This could be the cause for the observed differences in combination with a roof effect. Animals in the present study pecked > 12 times in 4 s in response to the stimuli followed by small amount of food already,
which prevents to detect differences, probably. In the same direction points the non-significant difference in responding between the groups during the test for latent inhibition. Even though the BEV group showed in previous phases (acquisition, extinction, retrieval) stronger responding to stimuli, animals in this group emitted less pecks during the test of latent inhibition, hinting at a small effect of the manipulation on attention paid to the context. However, group sizes were obviously very restricted what hindered to detect small effects of the manipulation. The same is true for the expected difference in context-specific responding between groups. We saw group responses that point to the expected direction: slightly larger effect sizes for the BEV group (table 1) and less responding in the test for latent inhibition (figure 7). However, differences between the groups before any experimental manipulation (in acquisition) also indicate, that sample sizes were too small for a between-group comparison. Additionally, the selected paradigm seems to be suboptimal, since we had a very high dropout of animals (~ 50%) due to non-differential responding between rewarded and non-rewarded stimuli in one of the phases. Therefore, we decided to no longer test animals in this task but further investigate the context-specificity of learning in a well-established within-subject design (study two).

2.4.3. Summary

In summary, we cannot draw any conclusion concerning the influence of expectancy violation on context-specificity of learning due to methodological issues, but saw pronounced context-specificity of acquisition as well as extinction memory once context relevance is experienced. As we were very impressed by the strong context-specificity, we decided to further investigate this effect in a less complex paradigm which would be suitable for further neuroscientific investigations (namely pharmacological manipulations of brain regions) while animals acquire and extinguish conditioned responding. The paradigm and behavioral results are described in the next chapter.
3. Context-specificity of acquisition and extinction in a within-subject design

3.1. Introduction

In the previous study (chapter one), we compared the context-specificity of acquisition and extinction memory as well as the influence of expectancy violation on the memory. While we could not draw any conclusion concerning the influence of expectancy violation, we observed context-specific memory for both acquired and extinguished responding. Furthermore, it seemed like that the amount of context-specificity is comparable high for both types of learning. However, conclusions from this study are somehow limited due to small sample size within the subgroups. We decided to try to replicate the findings in a within-subject design which offers stronger statistical power and the opportunity to combine the behavioral paradigm with pharmacological interventions. The final goal would be to gain insight if the acquisition and extinction of a response rely on the same neuronal mechanisms. The latter will be discussed in more detail in the discussion part of this study as well as in the general outlook at the end of this thesis.

In sum, we combined a well-established within-subject paradigm used in the lab (described in Lengersdorf et al., 2014b; Rescorla, 2008) with the paradigm described in chapter one. In the end, we introduced another stimulus in the extinction phase which was followed by food to observe the simultaneous acquisition of a response. Thus, we subjected animals to a sign-tracking paradigm, where the presentation of one stimulus was followed by food first (acquisition). Then, after a context change, this stimulus was no longer followed by food (extinction). Simultaneously, we introduced a second stimulus which was followed by food. Finally, we tested for responding to all stimuli in both contexts during the retrieval test.

Because we have observed strong context-specific responding for the stimulus to which responses were recently acquired in study one, we predicted also this time context-specificity for both acquired and extinguished responses. We expected stronger responding to the stimuli in their respective acquisition compared to the extinction context.
3.2. Materials and Methods

3.2.1. Subjects

Ten experimentally naive homing pigeons (Columba livia) served as subjects in this study. Two animals failed to show differential responding to Target vs. nonTarget stimuli at the end of the pre-training phase and were therefore excluded. Housing and feeding conditions were as described in 2.2.1.

3.2.2. Behavioral apparatus

Two operant chambers were used in this experiment, each measuring 34cmx34cmx50cm. At the back wall of each chamber, a LCD touch screen was mounted. Stimuli were presented at and key pecks registered from the touch screen. Each effective key peck produced an audible feedback click. Centrally below the monitor, a food hopper was placed and permitted access to food (grain) when intended. Each chamber was housed in a sound-attenuating shell. In one chamber (context one) the walls were lined with yellow wallpaper and loud speakers provided white noise, whereas in the other chamber (context two), the walls were painted red and brown noise was audible. Please note that the physical contexts were labeled as context one and context two and we do not use the classical ABA terminology, because of the within-subject design. All hardware was controlled by custom-written MATLAB code (The Mathworks, Natick, MA) using the Biopsychology Toolbox (Rose et al., 2008).

3.2.3. Procedure

The procedure was based on the one described in Lengersdorf et al. (2013). The experiment consisted as the one in chapter one of an acquisition, extinction and retrieval as well as a pre-training phase. Again, a sign tracking procedure was used. The sequence of events in one trial was the same as in study one (except the ITI and feeding times) and is depicted in figure 3. An overview of the stimuli and context in the learning phases is depicted in table 2.
3.2.3.1. Pre-Training

During pretraining, animals were habituated to the skinner boxes and confronted with the control stimuli (Target (+) and nonTarget (-)). This phase lasted four weeks and animals were tested daily in each context. In the first week, an autoshaping procedure was performed. After an ITI of 120 s the Target stimulus was presented for 4 s after which the food hopper offered 2 s access to food. In the second week, the nonTarget stimulus was introduced and its presentation was not followed by food. In the following two weeks, the ITI was stepwise decreased to 30 s while the amount of trials was increased up to 80 trials a session.

3.2.3.2. Phase 1/ Acquisition

In this phase, animals were confronted with a new stimulus in each context, respectively. Meaning that, stimulus CS1 was only presented in context one while CS2 was only presented in context two. Both were followed by food. In sum, animals performed 80 trials in each context daily. All stimuli occurred equally often. Stimulus presentation times, ITI and feeding times resemble the conditions at the end of the pre-training. When animals emitted at least one peck to the CSs in 80% of the trials in both contexts for three days in a row, animals were subjected to the extinction phase. However, this phase consisted of at least six daily sessions even when the described criterion was fulfilled earlier.

3.2.3.3. Phase2/ Extinction

During the extinction phase, the presentation of CS1 and CS2 was no longer followed by food. Importantly CS1 was presented in context two now while CS2 was

---

**Table 2: Overview of the paradigm in study two.** Rows depict stimuli in den different phases (columns). Colors indicate contexts.

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Phase</th>
<th>acquisition</th>
<th>extinction</th>
<th>retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS1+</td>
<td></td>
<td>CS1-</td>
<td>CS1-</td>
<td>CS1-</td>
</tr>
<tr>
<td>CS2+</td>
<td></td>
<td>CS2-</td>
<td>CS2-</td>
<td>CS2-</td>
</tr>
<tr>
<td>CS3+</td>
<td></td>
<td>CS3-</td>
<td>CS3-</td>
<td>CS3-</td>
</tr>
<tr>
<td>CS4+</td>
<td></td>
<td>CS4-</td>
<td>CS4-</td>
<td>CS4-</td>
</tr>
</tbody>
</table>
shown in context one. In addition, two new stimuli were introduced. CS3 was presented in context two and CS4 in context one. Their presentation was followed by food. Please note that the extinction phase took place on two different days for the particular stimuli. The sequence of contexts in which animals were tested was randomized.

3.2.3.4. Phase 3/ Retrieval

In the Retrieval phase, all four CS as well as Target and nonTarget were presented in each context on the same day, separated by 1h. All stimuli were presented twelve times while the Target was presented as often as all stimuli together to maintain animals’ motivation since all stimuli but the Target were not followed by food. Sample presentation and feeding times as well as ITI were identical as in the other phases. A session consisted of 90 trials.

3.2.4. Analyses

The absolute number of pecking in response to presented stimuli served as the dependent variable in this experiment. Due to the nature of the experimental design, only within-subject comparisons were performed, including repeated-measures analysis of variance (rmANOVA) with two within subject factors, and paired t-tests. All analyses were conducted in MATLAB (The Mathworks, Natick, MA; statistical toolbox).

3.3. Results

The presented results here follow the logic of study one. We first present the mean responses to the respective stimuli during the three phases (figure 8) as well as the difference in responding in context one and two (figure 9) and finally the responses to control stimuli across contexts (figure 10). As described in the introduction, we had two aims: replicating the effect of context-specificity of acquired responses as well as establishing a within-subject design for future combination with pharmacological interventions. Thus, we used a within-subject design where animals in phase one acquired responding to two stimuli (CS1 in context one and CS2 in context two), and extinguished responding to these stimuli in phase two in the respective other context. In the same phase, animals acquired responding to two novel stimuli (CS3 and CS4). Since CS1 and CS2 on the one hand and CS3 and CS4 on the other
were treated equivalently, we will present results averaged across these stimulus pairs (CS1 and CS2 = CSEx; CS3 and CS4 = CSAc).

3.3.1. Phase 1/ Acquisition

In the leftmost panel of figure 8 the mean responses to the CSEx (blue) and Target (red) in blocks of four trials in the acquisition phase are shown. The CSEx was introduced in this phase, and the presentation was consistently reinforced. Responding (pecking) was quickly established, i.e. in the first blocks, leading to a non-significant main effect of block when a one-way ANOVA is performed ($F(31) = 0.62$ $p = 0.941$). However, when compared to the Target stimulus (red line), post-hoc one-sample t-tests reveal a significant difference between Target and CSEx in the first three blocks only ($t(7) = -4.45$ $p = 0.003$; $t(7) = -3.73$ $p = 0.007$; $t(7) = -2.86$ $p = 0.024$), confirming an increase of responding to the CSEx from less responding compared to the Target to the same level as the Target.

3.3.2. Phase 2/ Extinction

During extinction (middle panel of figure 8), the presentation of CSEx was no longer followed by food. Accordingly, the amount of pecking decreased across the time course of phase two. Simultaneously, CSAc was newly introduced whose presentation was followed by food. As expected, an increase in responding can be observed. This observation was confirmed by a repeated measures ANOVA with two within-subject factors (stimulus and block) which yielded a significant main effect of block ($F(14,7)=2.91$ $p < 0.001$), along with a near-significant effect of stimulus ($F(1,7) = 4.54$ $p = 0.051$) and a significant interaction of stimulus and block ($F(14,1) = 12.05$ $p < 0.001$), reflecting an increase/decrease over time, more pecking for the CSAc than CSEx. In addition, the difference between stimuli was more pronounced at the end of the phase.

3.3.3. Phase 3/ Retrieval

In phase three, all CSs were presented in both contexts (accompanied by Target- and nonTargetstimuli) without subsequent reinforcement. The right panel of figure 8 shows the mean responses to stimuli to which responding was recently extinguished (blueish area) or acquired (reddish area). Dotted bars present the responses...
in the respective acquisition context. Left bars are associated with responding in the physical context one and right ones in context two.

A repeated measures ANOVA with two within-subject factors (stimulus and context) yielded a significant effect of context \((F(7,1) = 4.8 \ p = 0.006)\) but no effect of stimulus \((F(1,7) = 3.96 \ p = 0.185)\) and no significant interaction \((F(7,1) = 1.97 \ p = 0.132)\). This demonstrated more responding to stimuli in their respective acquisition context.

![Figure 8](image)

**Figure 8:** Mean amount of responding to stimuli across acquisition, extinction and retrieval. The leftmost panel depicts mean responses in blocks of four trials for all animals in the acquisition phase for the Target stimulus (red) and CS1 (blue). Central panel: Responses to the CS1 (blue) and CS2 (yellow) in the extinction phase. Rightmost panel: Responses in both contexts and to both CS in blocks of 12 trials are shown. Red shaded area stands for recently acquired responses while blue shaded area signify recently extinguished responses. Dotted bars show responding in the respective acquisition context. Error bars show Standard Errors of the Mean (SEM).

### 3.3.4. Difference in responding between context one and two

Like in the first study, we wanted to compare the amount of context-specificity for acquired and extinguished responding. Figure 9 shows the difference in responding between context one and two. A one sample t-test between the difference in responding yield a significant higher difference for acquired responses \((t(7) = -3.69 \ p =0.007)\), reflecting a more pronounced context-specificity for acquired responses.
3.3.5. Control stimuli

Two stimuli were presented in all phases and always (Target) or never (nonTarget) followed by food. To test for a possible induction of context-specificity, we compared the amount of responding between contexts for these stimuli, too. In figure 10, one can observe comparable high (Target) and low (nonTarget) responding in both contexts. A repeated measures ANOVA with two within-subject factors (stimulus and context) yield no significant effect of context ($F(7,1) = 0.06 \ p = 0.804$) but of stimulus ($F(1,7) = 65.3 \ p < 0.001$) and no significant interaction ($F(7,1) = 0.07 \ p = 0.798$), reflecting more responding to the Target than nonTarget but no difference for both stimuli between the two contexts.

![Figure 10: Context-independent responding to control stimuli. A) Mean responses to the Target (black) and nonTarget (grey) stimulus for both contexts. Errorbars show Standard Errors of the Mean (SEM).](image)
3.4. Discussion

In this study, we combined the experimental arrangement of study 1, i.e. that a response to one stimulus is acquired while responding to another is extinguished with a well-established within-subject renewal design (Lengersdorf et al., 2014b; Rescorla, 2008) We aimed at a replication of the observed context-specificity of acquired responses and collecting pilot data from a within subject-paradigm that allows for pharmacological manipulations during extinction/ acquisition.

We subjected pigeons to a within-subject renewal paradigm where responding to one stimulus was acquired in one context and extinguished in another one. During extinction, responding to a second stimulus was acquired. Finally, responding was tested in both contexts for both stimuli.

For the acquisition and extinction phase, results were as expected, i.e. animals increased responding to stimuli which were followed by food, and decreased responding to stimuli for which food was omitted. For the retrieval phase, we hypothesized for both responses, acquired and extinguished ones, an effect of context, i.e. we expected more responding in the respective acquisition context. Indeed, as can be seen in figure 8 (rightmost panel) animals responded more to the stimuli in the respective acquisition context (dotted bars). These results resemble exactly, what we have seen in study 1 of this thesis. However, when comparing the difference in responding between contexts (figure 9) we found that the difference was more pronounced for acquired than extinguished responses. Actually, we think that this effect is due to higher maximal responding to the stimulus to which responses were acquired (figure 8, right panel) and did not reflect a systematic effect. We did not observe a big difference in maximal responding for acquired and extinguished responses in study one. However, since more animals are about to be tested in this paradigm, one will see if this effect will be confirmed. Finally, we again observed context-independent responding to the control stimuli (figure 10), implicating that the learned relevance of context for specific stimuli is decisive if an association is acquired in context-specific way or not.

In the end, results of this experiment strongly resemble those of study one. Because, the implications of these results are discussed above and will be taken up in the final
discussion, we do not discuss them in detail here. In summary, the observed context-specific acquisition of responding contrasts with classical theories about context processing (Bouton, 2004) which stated that extinguished responding is more context-specific, because it is the second learned as well as an inhibitory association. Nevertheless, our results strongly support the attentional theory of context processing (Rosas et al., 2006), which predicts context-specific responding once ambiguity or context-relevance for a specific stimulus is experienced. This is exactly what we found.
4. Dynamic coding patterns in single units of the avian forebrain across three stages of learning – a special role for prefrontal areas in extinction

4.1. Introduction

In the general introduction, I gave an overview of the brain regions involved in learning. It was argued that similar regions (namely, amygdala, hippocampus and prefrontal cortex, among others) participate during acquisition and extinction of an association as well as in learning from appetitive and aversive outcomes. However, the predominant processes associated with these structures differ.

The amygdala is classically seen as the place for neuronal plasticity during (acquisition-) learning. A study investigating single unit activity in the dorsolateral amygdala (LAd) revealed for example, that the LAd is involved in the initiation and storage of (fear) memory since the activity of single neurons is correlated with behavioral changes during acquisition and keep their activity during extinction (Quirk et al., 1995). In addition, two subpopulations of neurons were identified, of which one (with short latency responses) is likely more involved in the initiation, whereas the other with long latency responses acts more on the consolidation of fear memory. Furthermore, a study investigating the changes in the neuronal network during development shed light on the causal mechanisms of the sustained memory trace despite extinction training (Gogolla et al., 2009). They could show that so called peri-neuronal nets in the amygdala develop at the same time as a qualitative change in the consequence of extinction learning occurs: extinction training in early life leads to erasure of fear memories while later extinction training does not prevent reoccurrence of fear. Furthermore, disruption of these peri-neuronal nets in adult animals leads to the erasure of the memory during extinction indicating the causal involvement of these nets in storing emotional memories.

The hippocampus in contrast is thought to be essentially involved in the encoding of context (Maren et al., 2013; Holland and Bouton, 1999), because hippocampal lesions lead to deficits in freezing during contextual fear conditioning (Phillips and LeDoux, 1992) and hippocampal neurons respond strongly to contextual or spatial cues. For example, so called place cells respond when the animals are located in specific spatial places (Moser et al., 2008) and their response profile is influenced by contextual cues (Smith and Mizumori, 2006a; Smith and Mizumori, 2006b).
However, hippocampal lesions do not necessarily lead to a complete blocking of contextual conditioning: Lesions made before conditioning (Wiltgen et al., 2006) or pre-exposure to the context before lesion (Young et al., 1995), allow for normal contextual conditioning. Thus, contextual cues are assumed to be stored not in the hippocampus itself but in a distributed network of the PFC and amygdala (Orsini et al., 2013; Orsini and Maren, 2012; Orsini et al., 2011) with the hippocampus having a decisive role in encoding contextual cues.

The clearest differentiation in regard to the involvement in acquisition or extinction, can probably be made for the prefrontal cortex (PFC), whose avian functional equivalent is investigated in this thesis. There are some studies showing the involvement of the PFC in first acquisition (Asaad et al., 1998; Rainer et al., 1998a; Rainer et al., 1998b; Puig and Miller, 2012a; Quirk et al., 1995), but the majority of studies opt for a special role of the PFC in extinction, i.e. when a former action has to be inhibited to act optimally: First, neuroimaging studies could show the involvement of the PFC during extinction in humans, namely, that activity in the PFC during extinction predicts the recall of extinction memory later on (Phelps et al., 2004; Milad et al., 2007). Lesion of the PFC block extinction (Lebron et al., 2004) and stimulation prevents conditioned fear respectively enhances extinction (Milad et al., 2004; Van den Oever et al., 2013). Finally, single neurons in the PFC show bursting behavior during extinction learning and elevated activity during extinction recall (Milad and Quirk, 2002; Burgos-Robles et al., 2007; Milad and Quirk, 2002).

In the following, I will concentrate on the PFC as a putative key player during extinction within the network and report studies investigating the role of the PFC in (extinction-) learning and its interaction with the amygdala and hippocampus. I will lay a focus on single unit recordings.

4.1.1. Single unit recordings during learning

A more and more popular approach for investigating the neuronal correlates of learning is the tracking and analysis of single unit activity on a trial-by-trial basis during learning (Czanner et al., 2008). This allows for a deeper analysis how the brain or its activity pattern gives rise to behavior. Dynamic changes in single neuron activity accompanying learning-related changes on the behavioral level have been described in various brain regions: supplementary and frontal eye field (Chen and
Wise, 1995a; Chen and Wise, 1995b), sensory cortices (Moran and Katz, 2014) prefrontal cortex (Asaad et al., 1998; Chen and Wise, 1995b; Histed et al., 2009; Loh et al., 2008; Pasupathy and Miller, 2005), hippocampus (Wirth et al., 2009; Wirth et al., 2003) striatum (Tremblay et al., 1998; Tremblay and Schultz, 2000; Pasupathy and Miller, 2005) and amygdala (Belova et al., 2008; Paton et al., 2006). And neuroimaging studies in humans indicate the same regions to be involved (Garrison et al., 2013).

A classic study already in 1998 recorded “Neuronal Activity in the Primate Prefrontal Cortex during Associative Learning” (Asaad et al., 1998). Two monkeys learned to make saccades in response to stimuli and the correct response was repeatedly reversed. Neurons represented the stimuli as well as the respective response during different epochs within the trials. Learning was reflected in a progressively earlier representation of the choice within the trial. The authors conclude, that the PFC represents all information needed to solve the task and is thus involved in learning stimulus-response associations.

However, comparable investigations regarding the neuronal dynamics during extinction learning are rare and concentrate on the amygdala and/or fear learning. Livneh and Paz (2012) recorded single unit activity in the amygdala while macaques were engaged in an association task with appetitive and aversive US comprising acquisition, extinction and retrieval. They found that different subpopulations were active during acquisition and extinction, but overlapping populations code for the association with appetitive and aversive outcomes. Another example is a recent study by (An et al., 2012), where single unit activity in the lateral amygdala was recorded during acquisition, extinction and reacquisition in a classical fear conditioning paradigm. They found two subpopulation of neurons: extinction resistant neurons showed an increased activity during acquisition, which outlasted the extinction stage, probably reflecting the stable trace of the fear memory throughout extinction. Extinction-susceptible neurons decreased their firing during extinction after elevated activity during acquisition. In addition, some of them quickly renewed the potentiated firing during reacquisition, exhibiting a neuronal correlate of rapid reacquisition or savings.
4.1.2. The prefrontal-amygdala-hippocampal network

Methodological innovations within the last decades now allow for experimental designs to investigate neuronal networks as well subpopulations of neurons that were not possible before. Multi-electrode arrays (MEAs) in combination with computer offering higher computational power now enable researchers the convenient acquisition and analyses of several hundred spike trains. Therefore, it is now feasible to record in parallel from different brain regions and investigate their interaction based on temporal activity patterns. In addition, the simultaneous application of drugs in specific brain region and single unit recordings can shed light on the involvement of specific receptors in producing differential firing rates and finally behavior. Lastly, optogenetic targeting and manipulation of different subtypes of neurons enable a finer resolution of functional circuits and make it possible to draw causal conclusions about the involvement of neurons in specific functions. This contributed heavily to our understanding of neuronal networks and their interaction to produce adapted behavior. Also the investigation of the neuronal mechanisms of (extinction-) learning profited of this as the field focusses now on the strongly interacting network of amygdala, PFC and hippocampus.

A study combining the application of pharmacological agents in parallel to single unit recording was performed by Puig and colleagues (Puig and Miller, 2012a). Monkeys learned new stimulus-saccade associations and performed on familiar ones while the activity of single units in the prefrontal cortex was recorded. Additionally, a dopamine receptor 1 (D1) antagonist was administered in a block wise fashion. It could be shown that the inhibition of the D1 receptor in the prefrontal cortex prevents the learning of new associations while familiar ones are unaffected. Concurrently the antagonist eliminated changes in neuronal activity which were seen during learning under drug-free conditions. This emphasizes the role of the prefrontal cortex and dopamine in learning of new stimulus-response associations. Similar, an study investigated the interaction between the mentioned key structures and found that the hippocampus is gating the input from the amygdala to the prefrontal cortex for the expression of fear (Sotres-Bayon et al., 2012). They subjected rats to a fear conditioning paradigm and inactivated either the hippocampus or basolateral amygdala (BLA) with muscimol while recording tone evoked single unit
responses in the prelimbic cortex. While inactivation of the BLA decreased the activity of projection cells and decreased evoked responses to the CS, inactivation of the hippocampus decreased the activity in interneurons and intensified conditioned responses of the PL in extinguished but not in conditioned only rats. The authors conclude that this suggests a form of interaction, that the hippocampus is gating amygdala-based fear expression after extinction.

An influential study (Herry et al., 2008) investigated the coding properties of single units in the BLA during acquisition, extinction and renewal of a conditioned fear response. They found two populations of neurons, that were selectively active during acquisition or extinction (“fear” and “extinction neurons“) and showed different connection patterns with the prefrontal cortex and hippocampus: fear but not extinction neurons received input from the hippocampus whereas extinction neurons were reciprocally connected with PFC and fear neurons received input from but not projected to PFC. In addition the activity patterns of fear and extinction neurons mirrored the behavioral expression or inhibition of fear. In conclusion, the authors argued, that the balance of activity in fear and extinction neurons in the BLA allow for rapid switches between low and high fear states and the respective activity mediates the consolidation of fear or extinction memory. A similar illustration of top-down-control of the PFC comes from an investigation showing that the stimulation of the PFC leads to an inhibition of a conditioned response in neurons of the central nucleus of the amygdala (Rosenkranz et al., 2003); a process which is modulated by dopamine (Rosenkranz and Grace, 2003).

Finally, optogenetic targeting of parvalbumin positive interneurons revealed that these neurons participate in the expression of learned fear (Courtin et al., 2014) and that their optogenetic stimulation or inhibition leads to suppression or expression of this fear. In addition, theta oscillations in the ventromedial PFC as well as disinhibition of cells projecting to the BLA modulate the expression of fear, emphasizing the role of a PFC-amygdala interaction in emotional memory. Related to this, optogenetic targeting and manipulation of the network revealed that the extinction of conditioned fear results in a decreased synaptic efficiency from the PFC to BLA interneurons (Cho et al., 2013).
In summary, amygdala-prefrontal-hippocampal interactions built the backbone for learning related plasticity in the brain (Likhtik et al., 2005). Dopamine as a modulating neurotransmitter as well as interneurons of different subtypes due to their strong influence on network activity play an important role. Even though most investigations on this topic again focused on fear learning, the first studies concentrating on reward-based learning point in a similar direction. However, more research is needed to elucidate similar mechanisms for learning from positive consequences. Nevertheless, the review of the interaction pattern of amygdala, hippocampus and PFC further emphasize the crucial role of the PFC as central hub for integrating inputs from different sources, as it is necessary during extinction learning.

4.1.3. The prefrontal cortex in learning

For a successful adaptation to changes in the environment, information from different sources have to be integrated and the prefrontal cortex (PFC) has been implicated to undertake this function (Miller and Cohen, 2001). Because the process of extinction learning is associated with contradicting or ambiguous information about specific stimuli, contextual or in general more information about the situation is integrated into the memory trace to optimally act on a task (see behavioral studies of this thesis or (Bouton, 2004)). This means in addition that during extinction learning the PFC receives an outstanding role when integrating information about alternative meanings of stimuli, contexts and response options. And finally the PFC selects the appropriate response (Euston et al., 2012).

4.1.3.1. Infralimbic and prelimbic cortex

Two regions in the PFC have been implicated in mediating acquisition and extinction of conditioned fear: the infralimbic (IL) and prelimbic cortex (PL). And their assumed functions complement each other: The IL is thought to inhibit the expression of fear after extinction by projecting onto interneurons in the amygdala (Likhtik et al., 2008; Rosenkranz and Grace, 2002), whereas the PL is acting on fear expression during acquisition and retrieval and projecting to neurons in the amygdala (Likhtik et al., 2005; Burgos-Robles et al., 2009). Similar results were obtained in imaging studies of the human brain, where the activity within the ven-
tromedial prefrontal cortex (vmPFV, human homolog of IL) during extinction predicts extinction success (Phelps et al., 2004; Milad et al., 2007) and noradrenergic stimulation enhances extinction learning as well as increases vmPFC activation (Lissek et al., 2015). Furthermore, single unit recordings within the IL report elevated firing rates in response to conditioned tones during extinction (Holmes et al., 2012; Chang et al., 2010). Finally, a very recent study shows that silencing the activity of glutamatergic neurons in the infralimbic cortex during extinction does not influence extinction learning but storage of extinction memory (Do-Monte et al., 2015). Similar results have been found in the appetitive domain: Activation of interneurons in the prelimbic cortex reduced the overall activity in this region and was accompanied by enhanced extinction of reward seeking behavior (Sparta et al., 2014). A study using in vivo oxygen amperometry during reward-based learning showed increased activity during early acquisition and extinction in the infralimbic cortex, strengthening the role for the prefrontal cortex for suppressing ineffective actions during learning (Francois et al., 2012) and NMDR receptor agonist application as well as BDNF in the IL enhances extinction (Peters and De Vries, 2013; Otis et al., 2014). Finally, the IL has been shown to regulate food intake behavior controlled by the nucleus accumbens (Richard and Berridge, 2013) and more general in the extinction of drug taking behavior (Millan et al., 2011). However, there are also studies conducting single unit recording in the IL indicating that the relationship between single-unit activity and extinction performance does not hold under all experimental condition (Fitzgerald et al., 2014; Chang et al., 2010; Willcocks and McNally, 2013). Because knowledge about the involvement of the prefrontal cortex in appetitive behavior is extremely restricted outside the drug-addiction field, we were interested if we could find a similar involvement of the PFC in reward-related learning and single unit responses resembling those during the acquisition and extinction of fear responses in PL and IL.

4.1.4. Rationale and hypotheses

To investigate the neuronal dynamics during learning with a focus on changes associated with extinction, we designed a task which allowed for the recording of single unit activity across three stages of learning. As pointed out above, the IL region of the PFC is assumed to play a major role in the inhibition of previous behavior under changing conditions in appetitive and aversive tasks. However, the
neuronal correlates on a single unit basis with high temporal resolution during extinction remains to be determined. Following this, we decided to investigate the possible neuronal correlate during extinction in an operant and appetitive task where learning spans several tens of trials and allows for a trial-by-trial analyses of neuronal dynamics while behavior changes. In detail, we trained pigeons on a single-interval-forced-choice task (SIFC) where animals learned to execute a left or right peck choice in response to novel visual stimuli to obtain food reward. One experimental session spans three learning stages, where animals acquire the correct response by trial-and error learning (acquisition), extinguish it because of the omission of the food reward (extinction) and then reacquire it because of the de novo reinforcement. Simultaneously, we recorded single-unit-activity in the avian functional analogue of the prefrontal cortex: Nidopallium Caudolaterale (NCL).

4.2. Materials and Methods

4.2.1. Subjects

In this study, five pigeons were tested and electrophysiological recordings were made. Animals were tested in another visual discrimination task before. However, different stimulus sets were used to exclude perturbation of the present experiment. Animals were housed in aviaries in groups of eight before and individually in wire-mesh cages after electrode implantation. Remaining housing and feeding conditions were as described in study one (Subjects 2.2.1.)

4.2.2. Behavioral apparatus

Behavioral testing and recording took place in an operant chamber (34cmx34cmx50cm). Three pecking keys were inserted into the back wall of the chamber in a row (see figure 11). Pecking was registered and led to an audible feedback click. Below the central key, a food hopper provided access to food, when intended. All hardware was controlled by custom-written MATLAB code (The Mathworks, Natick, MA;) using the Biopsychology toolbox (Rose et al., 2008). For a detailed description of the recording setup see Starosta et al. (2014).

4.2.3. Procedure

Before each recording session, the screw of the implanted electrode-array was turned to lower the electrodes (see details below) and reach new cells to record
from. Electrodes were lowered one hour before recordings started to allow the tissue to expand again and assure stable recordings throughout the session. In between, animals were placed in their home cages again. For details of the training procedure to prepare animals to perform the final task see (Starosta et al., 2014).

4.2.4. Single-Interval-Forced-Choice task across three learning stages

In general, each testing session consisted of three learning stages (acquisition, extinction, and reacquisition). The animals had to learn to make a side peck to left or right in response to stimuli they had never seen before (acquisition). Then responses to one randomly chosen novel stimulus were no longer reinforced (extinction) and finally reinforced again (reacquisition).

The sequence of events within one trial is shown in figure 11. After an ITI of 5 s the central pecking key was illuminated green and animals had to emit three responses to it for initialization of the trial. If they did not, the trial was aborted and the ITI started (initialization omission). After the initialization, one out of four sample stimuli was presented for a fixed time of 2 s (sample phase). The animals had to emit at least one response; otherwise, the trial was aborted and counted as sample omission. After sample presentation the central key was illuminated green again and the animals had to peck at it once (confirmation), before the two side keys were illuminated green and animals had to make their choice to the left or right (choice.

**Figure 11: Behavioral task of study three.** Each trial consisted of five phases. After an ITI of 5 s (1), an initialization stimulus is presented at the central pecking key (2). After three pecks, one out of four sample stimuli appeared for 2 s (3). If animals pecked at least once, the confirmation stimulus (4) was presented until the animal pecked once (within 3 s). Then, illumination of the two side keys followed for 3 s or until animals made their choice (5). See text for details. In the right corner example stimulus pairs are shown. Adopted from Starosta et al., (2014).
phase). If they did not choose one of the side keys, it was counted as a choice omission. In general, a correct choice was rewarded with 2 s access to food, while an incorrect response was punished with a mild time out (2 s lights off). Correct choice to novel stimuli were always rewarded and punished (except to the one stimulus during extinction) while correct choices to familiar stimuli were rewarded in 70% of the cases. Incorrect choices always led to punishment (except to the one stimulus during extinction).

Within one session, for the first 20 trials, only two familiar stimuli (meaning that animals knew if to make a left or right choice, henceforth FS), were presented. After that, two novel stimuli (NS) were introduced. One familiar and one novel stimulus was associated with a left peck (henceforth FS1 and NS1) while the other familiar and novel stimulus were associated with a right peck (henceforth FS2 and NS2) in the choice phase. Proportions of NS and FS were equal and the sequence of stimuli was pseudo-randomized across trials. After 20 trials, novel stimuli were introduced and animals had to learn by trial and error how to respond (acquisition). When animals reached a predefined learning criterion (> 85% correct responses within the last 30 trials) and at least 120 trials were completed in this stage, one of the NS was randomly chosen and choice responses to it were no longer reinforced (no reward, no punishment). This stimulus will be termed extinction stimulus (ES) from now on. Please see table 3 for reinforcement conditions and performance criteria in the different stages. When performance regarding this stimulus dropped (extinction) to less than 65% correct responses within the last 30 trials, the extinction stage ended and correct responses were reinforced again (reacquisition). After animals performed again >85% correct, the session was terminated.

Table 3: Overview of reinforcement conditions and criteria in the three learning stages. Adopted from Starosta et al., (2014).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition</td>
<td>until performance for all stimuli &gt;85%</td>
</tr>
<tr>
<td></td>
<td>familiar cues - reward probability: 0.7</td>
</tr>
<tr>
<td></td>
<td>novel cues - reward probability: 1</td>
</tr>
<tr>
<td>Extinction</td>
<td>until performance for extinction-stimulus &gt;65%</td>
</tr>
<tr>
<td></td>
<td>the response to one novel, randomly chosen, cue is no longer reinforced</td>
</tr>
<tr>
<td>Reacquisition</td>
<td>as during acquisition, all responses are reinforced</td>
</tr>
</tbody>
</table>
4.2.5. Surgery

When animals performed repeatedly the final SIFC task including all three learning stages, they were implanted with a custom-built microarray, which is described in detail below. As initial anesthesia animals were injected with a ketamine-xylacin combination (7:3 units, 0.15ml/100g body weight) while anesthesia was maintained with isoflurane inhalation. When the animal was anesthetized, the feathers on the head were cut. It was placed in a stereotaxic apparatus and the scalp was cut and pulled sideways. Above the respective region of interest, a small hole was drilled into the skull and the dura was removed. Electrodes were then lowered into the brain. The hole was covered with vaseline and above that light-curing dental cement was applied. Another small hole was drilled into the bone above the cerebellum to place a silver ball connected to the array under the bone as ground. Six to eight micro-screws were placed into the skull to anchor the microarray with dental cement to the head. The wound was sutured and local antibiotics (tyrosur powder) were applied. Animals received analgetics (Caprofen. 10 mg/kg) for a minimum of two consecutive days after surgery and were allowed to recover for two weeks before testing started.

4.2.6. Electrophysiology

Signals were recorded via sixteen electrodes in a custom built microarray (see 4.2.6.1), which was connected to a preamplifier (npi electronic GmbH, Germany). Signals were 1000x amplified and prefiltered online (0.5 – 5Hz) by a difference amplifier (npi electronic GmbH, Germany) and digitized using an analog-to-digital converter (power 1401 A/D system, Cambridge Electronic Design (CED), UK) with a sampling rate of 16-20 kHz. The raw data was stored with Spike2 Version 7 (CED) for offline analysis.

4.2.6.1. Microarray

The microarray was built on the one described in (Bilkey and Muir, 1999; Bilkey et al., 2003). However, a quite extensive part of my PhD-work consisted of improving the actual recording technique including the array, so that I will describe the modifications in more detail. First, the number of electrode wires (formvar-insulated nichrome wires, 40um), was doubled leading to sixteen electrodes in one array. This required using a thicker guide cannula and larger micro-connectors.
(Ginder Scientific, Nepean, CA, USA). The connectors provided the opportunity to firmly connect to the headstage, leading to extraordinary improvements in both signal quality and minimization of movement-related artifacts. Additionally, we adopted a gold-plating protocol from colleagues from Münster University (Dr. Thomas Seidenbecher) to decrease electrode impedance from ~1 MΩ to values <30 kΩ, and therefore reducing background noise.

4.2.6.2. Quality control

Recording single unit activity from freely moving animals poses several challenges and requires sophisticated quality control. We focused on three parameters: 1) sufficient Signal-to-Noise-Ratio; 2) clear distinction of single units; 3) stable recording over the time course of a recording session.

4.2.6.3. Spike sorting

Spike sorting procedure is described in detail in Starosta et al. (2014). First, we filtered the raw data offline (300-5000 Hz) and then extracted spikes via a threshold procedure and PCA analyses implemented in Spike2 Version 7 (CED) as well as custom written MATLAB code (The Mathworks, Natick, MA; mlib toolbox for the analysis of spike data, Maik Stüttgen MATLAB central file exchange). We applied certain criteria to include a unit to the final data set. First, plotting all recorded waveforms should yield a clear average waveform and no sign of overlaying waveforms from other units. Second, waveforms should form a clear separable cluster in PCA space and peak waveform amplitudes should follow a normal distribution with no sign of false positive or negative classification. Finally, all units which fulfilled these criteria and had a Signal-to-Noise-Ratio (SNR) of at least two (reflecting that signal and noise are separated by eight standard deviations) were used for further analyses. This was true for 148 units.

4.2.6.4. Stability of recordings

To assure good signal quality and stable recording throughout the long-lasting recording sessions, we applied some post-hoc criterion to exclude units from further analyses. First, we computed the peak-to-peak amplitude of every waveform in every session and checked for a systematic increase or decrease. Second, we divided each session in five equal parts and compared the peak-to-peak amplitudes of the
respective parts to each other with a paired t-test. Since results of significance tests are heavily dependent on sample size and we compared up to 100,000 waveforms from every part of the session to each other, we decided to include neurons for which effect sizes did not exceed small values (Hedges’ g < 0.2), regardless of statistical significance.

4.2.6.5. Analyses

All analyses were performed in MATLAB (The Mathworks, Natick, MA; statistical toolbox and mlib toolbox for analyzing spike data; Maik Stüttgen, MATLAB central file exchange).

4.2.6.5.1. Behavioral analyses

On the behavioral level, we compared the average number of trials in each stage to reach criterion as well as reaction times and pecks towards the sample stimulus, using a Friedman Test and Wilcoxson Ranked Sum Test.

4.2.6.5.2. Electrophysiology

The baseline firing rate was calculated over the last two seconds of the ITI, i.e. the two seconds before the initialization stimulus was presented. In addition, we computed peri-stimulus-time histograms for the four different stimuli on a trial-by-trial basis as well as for the three different stages. We convolved them with an exponentially modified Gaussian kernel with a Standard Deviation (SD) of 100 ms and a time constant of 100 ms to obtain spike density functions.

4.2.6.5.3. Clustering

To cluster the units into two groups, we used a k-means algorithm implemented in MATLAB based on their waveform (full width half-maximum time for the first and second peak of the waveforms).

4.2.6.5.4. Modulation of firing rates

To test for modulation of the firing rate during movement execution (key pecking), we constructed PPTHs (peri-peak-time-histograms) as described in Lengersdorf et al. (2014). Each PPTH holds two bins (50ms each) before and after each key peck.
delivered to the initialization stimulus. To detect any change in firing rate, we used the non-parametric Chi-squared test (deviation from a uniform distribution).

We used non-parametric test to compare raw spike counts during stimulus presentation (Wilcoxon Ranked Sum Test for two and Kruskal Wallis Test for more than two groups).

4.2.6.5.5. Neuro-Behavioral Correlation

To compare changes in neuronal activity in relation to changes on the behavioral level (learning), we computed the correlation (Pearson’s r) between the behavioral performance and raw spike counts during stimulus presentation (2 s) as it was performed in similar experiment in non-human primates (see Wirth et al, 2003 for an example). Correlations were computed for the complete session as well as for the three stages separately.

4.2.6.5.6. Selectivity Index

As a measurement of differentiation between the two novel stimuli, we calculated a selectivity index (SI) as it was described previously (Asaad et al., 1998) The SI is thereby purely the difference between the firing rate to stimulus one and two divided by the sum of the firing rates. We choose the novel stimuli only, because responses to the stimuli is what has to be learned during the experiment.

4.2.6.5.7. Correlations between neurons

We computed cross-correlation histograms (peri-spike-histograms) with 1 ms bins and compared raw spike counts of one unit between bins before and after an action potential of the respective other unit (Wilcoxon Signed Rank Test).

4.3. Results

Overall, behavioral and neuronal data from five birds in twenty experimental sessions was analyzed. Sessions were included if animals showed stable performance throughout the session for familiar stimuli and reached at the least the criterion of completed extinction. Therefore, in three of the sessions or ten units, data was analyzed for the acquisition and extinction stage, only.
4.3.1. Behavioral results

4.3.1.1. Example session

Figure 12A depicts behavioral performance of one animal in an example session. Colored lines show percentage of correct responding for different stimuli as a moving average over the last 120 trials. The percentage of correct responses started quite low and was continuously increasing during acquisition for novel stimulus 2 (NS2, magenta line) while performance for novel stimulus 1 was high from the beginning (NS1, cyan line). On average, performance started at change level (black line). When the animal performed for both NS > 85% correct, NS2 was chosen by change to undergo extinction. This resulted in decreasing performance during extinction. When performance dropped under 65%, responses were reinforced again and performance increased rapidly. Performance for FS was high throughout the session (red and blue line).

4.3.1.2. Group analyses

To assess the general suitability of the behavioral paradigm, we computed the average amount of trials animals needed to reach criterion in each learning stage. The results are depicted in figure 12B. On average (across all sessions and animals), animals performed 368 trials (Standard Error of the Mean (SEM): 38; range: 120-743) in the acquisition stage, 167 trials (SEM: 9; range 50-202) in extinction and 90 (SEM: 7, range: 20-177) in reacquisition before they reached the respective criterion. Note that actual length of the learning stage could be somewhat higher because we set the minimum number of trials in each stage to 120. A repeated measures ANOVA revealed a significant effect of learning stage ($F(22) = 33.46; p < 0.001$). Post-hoc-test revealed that the number of trials in each stage differed significantly between all stages (acquisition vs extinction: $t(22) = 4.8; p < 0.001$; acquisition vs. reacquisition: $t(22) = 6.8; p < 0.001$; extinction vs. reacquisition: $t(22) = 4.9; p < 0.001$). Concerning reaction times and pecks on the stimulus, we observed stable performance levels across stimuli and sessions with the exception of the ES in the extinction and reacquisition stage. A Friedman Test however revealed significant differences in all stages for both amount of pecking and reaction times which was mainly driven by faster reaction times and more pecking to the FS1 (red line)
and a general increase in reaction times at the end of the session. However, in absolute numbers, this effect was relatively small (range of 4.6 – 5.2 pecks per stimulus and reaction times between 0.92-1.02 s) and will not be investigated in more detail. In extinction the amount of pecking was clearly reduced for the ES compared to all other stimuli (extinction mean: 3.9 vs. 5.2; 4.8 and 4.7 pecks; reacquisition mean: 3.6 vs. 5.2; 4.8 and 4.7 pecks). The post-hoc paired t-test confirmed this observation for both stages (extinction: $t(22) = 4.5; p < 0.001; t(22) = 3.1; p < 0.001; t(22) = 2.3; p < 0.001$; reacquisition: $t(22) = 5.3; p < 0.001; t(22) = 4.6; p < 0.001; t(22) = 4.7; p < 0.001$).

**Figure 12: Behavioral Analyses**

A) Example session of one animal. Percent correct responses as a moving average of 120 trials across the session are shown. Performance increases (acquisition), decreases (extinction) and increases again (reacquisition) for novel stimuli (magenta and black line). Performance for familiar stimuli is high throughout the session (blue, red line). Adopted from Starosta et al., (2014).

B) Mean trials per stage needed to reach predefined performance criterion (> 85% correct for acquisition/ reacquisition, < 65% for extinction). Amount of trials is decreasing from acquisition to extinction and from extinction to reacquisition.

C) Mean pecks on stimulus for familiar and novel stimuli split of for learning stages (ac = acquisition; ex = extinction; re = reacquisition).

D) As in C but for reaction times (choice).

In summary, behavioral results show that animals were able to repeatedly acquire, extinguish and reacquire a response to a stimulus within one experimental session.
Performance was extremely stable across sessions, learning stages and stimuli with the exception of decreasing performance for the ES in the extinction stage in form of less correct responding and less amount of pecking in response to this stimulus. However, this was intended by experimental manipulation of reinforcement conditions.

4.3.2. Electrophysiological results

Overall, 148 single units from the NCL were recorded. Units were not selected for any modulation initially, but analyses focused on dynamical changes across learning stages, where stimulus modulation could be expected to develop later in the session.

4.3.2.1. Stability of recordings

As described in the methods section, we wanted to exclude units, which showed no stable firing pattern or waveform across the session and computed effect sizes for the change in waveforms. Distribution of effects sizes are shown in figure 13A. Based on the criterion to exclude all units for which effect sizes exceed 0.2, we excluded 24 units from the sample which yield a group of 124 units for further analyses.

![Graph A](image)

**Figure 13:** Exclusion criteria for recorded units and measurable effects of methodological improvement. A) Frequency of effect sizes (hedges g) for the comparison of waveforms across the session. Units with an effect size $>0.2$ were discarded from the data sample. B) Amount of units recorded per session in the present study in comparison to a previous study (Starosta et al., 2013). Errorbars show Standard Error of the Mean (SEM). The yield of every session was on average three times higher in the present study.
4.3.2.2. Methodological improvements

As described earlier, a substantial part of my PhD work consisted of methodological advancements, thus I will shortly summarize their measurable effects. Briefly, we doubled the number of electrodes in the microarray and reconstructed it as well as applied a gold-plating protocol for the electrodes. These improvements allowed us now to record from more neurons per session when compared to previous studies (mean: 5.9 units vs. 1.8 units; see figure 13B). This means, that not only the doubled amount of electrodes increased the yield of the session (in this case, 3.6 units per session would be expected), but the gold plating improved the situation additionally.

4.3.2.3. Electrophysiological properties of NCL neurons

The mean spontaneous firing rate of all recorded units was 3.4 Hz (range 0.03Hz – 40.3Hz) and therefore somewhat higher than described before (Starosta et al, 2013). Figure 14A shows the mean width of the first phase of every mean waveform plotted against the second phase. This yielded two discernible clusters: one larger cluster (70% of the units, green) with broader waveforms and a smaller one (30%, red) with thin waveforms (putative inhibitory interneurons, type III neurons in (Kröner et al., 2002). Thus the average spike waveform was thinner for the smaller cluster (C). The proportion of this smaller cluster was higher as in previous studies (6% in Starosta et al., 2013 and 21% in a study by Nils Kasties, personal communication).

In figure 14B the spike width of the second phase is plotted against the firing rate of the units. On average, the spontaneous firing rate for the broad-spiking cluster was lower than for the thin-spiking cluster (mean: 1.9 Hz vs. 7.4 Hz). Figure 14D depicts the distribution of spike widths for the second peak. One can observe a bimodal distribution which supported the classification into two clusters. Together with the higher average firing rate, this is further support for the classification of the smaller cluster as fast spiking interneurons. The higher firing rate of the smaller cluster and the higher incidence of these neurons in comparison to previous studies explains the overall higher spontaneous firing rate in this data set compared to what was described before. It is likely, that the difference resulted from the applied gold-plating protocol also. On average, fast-spiking units do have smaller waveform amplitudes (see figure 14C) and are therefore more likely to be detected with a decreased noise level (a consequence of gold plating).
Due to the methodological improvements described above, we were able to record from groups of neurons simultaneously and could therefore analyze the interaction of neurons. If the smaller cluster described above displays a group of inhibitory interneurons, this should be observable in cross-correlograms of two inhibitory connected neurons close to each other. Indeed, we found that 10 (24%) of the neurons classified as interneurons show signs of inhibition (decreased activity after the other neurons emitted an action potential) with at least one simultaneously recorded unit in the cross-correlogram. An example is shown in figure 14E. A comparison between the first ms before and the one after yield a significant difference (signed rank = 6.5; p < 0.005, Wilcoxon Signed Rank Test). In addition, we found that 8 neurons have an excitatory connection like the two displayed in figure 14F (z = -2.7; signed rank = 375; p < 0.005, Wilcoxon Signed Rank Test).

Figure 14: Waveform clustering
A) Width of the first peak of every waveform is plotted against the width of the second peak. Two clusters were identified with a k-means algorithm and color-coded (green: cluster one; red: cluster two). B) Width of the second peak is plotted against the firing rate of the units. The smaller cluster (red) has a higher firing rate than the larger cluster. C) Mean waveform of the two clusters. The small cluster has on average thinner waveforms with a smaller amplitude. D) Histogram of the widths of the second peak shows a bimodal distribution. E) Example cross-correlogram of two units from different clusters. Dotted red line displays the time point of a spike by the red unit. It consists of a reciprocal connection. F) As in E but for two units of the same cluster. An excitatory connection is observed.
4.3.2.4. Motor modulation

The chi-squared test (see materials and methods) testing for any motor modulation of the firing rate by key pecking revealed that 44 of the 124 units (35%) showed a significant modulation ±100ms around pecking. An example is displayed in figure 15. The proportion of units with significant motor-modulation matches the ones described earlier (41% in Starosta et al., (2013); 27 % in Lengersdorf et al., (2014)).

4.3.2.5. Sample Phase

The focus of this study was the investigation of dynamical changes of single unit activity during learning. Because the learned association should be predominantly expressed during stimulus presentation, analyses were restricted to the sample phase of the trial.

4.3.2.5.1. General stimulus modulation

Of the 124 units, 93 (75%) exhibited significant modulation during stimulus presentation compared to baseline (p < 0.05, Wilcoxon Ranksum Test, across all learning stages). 2/3 of the units (58) increased while 1/3 units decreased their activity (35). Figure 16 displays two neurons decreasing (A, B) and two increasing (C, D) their firing rate during stimulus presentation. In addition to the general modulation in the sample phase compared to baseline, neurons shown in B-D were also stimulus-modulated, meaning that the response to at least one of the stimuli was significantly different from that of another. We found that over all stages, 70 units (56%) showed stimulus-modulated activity (p < 0.05, Kruskal Wallis Test). The unit in B also differentiated between stimuli which were associated with a left peck (NS1 and FS1)
or a right peck (NS2 and FS2) while neuron in C differentiated between novel (cyan, magenta) and familiar (green, red) stimuli. Overall, 50 (46%) neurons showed a differentiation between left and right while again 50 differentiated between old and new stimuli. However, it were not exactly the same neurons which differentiated between old and new and left and right. The overlap was 52% (26 units). Finally, the neuron depicted in D was clearly stimulus-modulated with a preference for the FS1 and NS2, which do not have any obvious similarity like novelty or associated response.

Figure 16: Baseline and stimulus-modulated activity of four example units. A) SDFs of an example NCL unit with significantly decreased activity during sample presentation for all stimuli (color codes). SDFs were smoothed by an exponentially modified Gaussian kernel (see material and methods). B) As in A) but for a unit with decreased activity compared to baseline and differentiation between stimuli associated with left and right responses (magenta, red). C) As in A, but for a unit which increased its activity during sample presentation and differentiating between all four stimuli as well as between novel (cyan, magenta) and familiar stimuli (green, red). D) As in A; Unit showed increasing activity during sample presentation, differentiated between all four stimuli with a preference for NS2 and FS1.
4.3.2.5.2. Stimulus modulation across learning stages

Since the focus of this study was the investigation of neuronal firing changes across learning stages, we separately tested for stimulus modulation for different learning stages (acquisition, extinction and reacquisition). Here, we tested only for differentiation between the two novel stimuli. Overall, 56% of the units (70) differentiated between the two novel stimuli (see figure 16A). Analyses for different stages revealed that out of the 124 recorded neurons, 53 (41%), 47 (36%) and 37 (29%) neurons showed differences in firing between stimuli during acquisition, extinction and reacquisition stages, respectively. This suggests a decreasing stimulus selectivity during the time course of the session. However, as seen in figure 12 the average amount of trials in each stage was significantly different (decreasing from acquisition to extinction and again from extinction to reacquisition), which in turn affects statistical power to detect a modulation, leading to a systematic underestimation of stimulus modulation in extinction and reacquisition in comparison to acquisition. Thus, we randomly selected from every stage as many trials as were available for the shortest stage. This led to comparable number of units showing stimulus related activity in the different stages; acquisition: 36 (27%); extinction: 31 (25%); reacquisition: 32 (26%) (see figure 17B). Please note, that this does not sum up to 100% or 70 modulated neurons because some units were differentiating in more than one stage. In detail, the pattern that approximately one third of the units differentiate in each stage could be due to several factors: first, it could be that the same neurons differentiated across all three learning stages, second, there may be neurons which differentiate only during a specific stage, or a combination of the two. To see what holds for the population recorded here, we split up the population of neurons differentiating in one stage according to their behavior during the other stages. This revealed that most of the cells (34 or 56% of the 70 modulated neurons) differentiated between stimuli in one of the three stages only, while a smaller group of neurons shows differentiation in two or all stages (11 units or 18% and 16 units or 26%), implying that different subpopulations of the NCL are involved when a response is acquired or extinguished in different learning stages (figure 17C). When examining for which stage neurons are mainly specialized, we found that out of the 34 units 38% (13 units) / 41% (14 units) differentiated only in the acquisition / reacquisition stage while 21% (7 units) showed differential responding in the extinction stage only (figure 17D). Figure 25 depicts a neuron which differentiated
between the two novel stimuli in extinction only, while the neuron in C shows the opposite pattern: differentiating in the acquisition and reacquisition stage. The results until now show that neurons in the NCL code for and differentiate between stimuli across different learning stages and that roughly the same number of neurons showed differential responding in each stage. However, most of the units were specialized in a way that they differentiated in one learning stage only.

**Figure 17**: Percentage of neurons differentiating between novel stimuli in different learning stages. A) 56% (70 units) of all recorded neurons (124) differentiate in at least one stage between NS. Number within the diagram shows absolute number of neurons. B) In all three stages approximately 30% of the units differentiate. C) The majority of units which differentiated in at least one stage (70), differentiate in one stage only (34 units or 56%). D) Units which differentiated in one stage only, are less for the extinction stage (7 out of 34, 21%).

4.3.2.5.3. Neuro-Behavioral-Correlation

To investigate the dynamic changes in the firing patterns of single neurons on a finer time scale and relate it to changes on the behavioral level during learning, we computed the correlation between neuronal firing and behavioral performance across the experimental session separately for each stage for the two novel stimuli. Figure 18 shows the distribution of correlation coefficients for the whole session.
(upper row) and for acquisition, extinction and reacquisition separated (rows two to four). The left column depicts the data for the stimulus, which was selected as extinction stimulus (ES) while the right column holds data for the other novel stimulus. Please note, that the ES was randomly selected every session and the columns therefore combine stimuli associated with both left and right key pecks. Yellow bars show all units, while green bars show units with significant correlations only. Red bars depict units for which activity during presentation of the respective stimulus correlated significantly with behavior but the activity in response to a control condition did not correlate with behavior. We choose for different learning stages/ stimuli the following control conditions: For acquisition, we chose for both novel stimuli the respective familiar stimulus associated with the same side response as control, i.e. that for NS1 (associated with a left peck) we choose FS1 (also associated with a left peck) and correlated the activity with behavioral performance. If this did not yield a significant result, we classified the unit as involved in the learning process, since the activity chances were not seen for a familiar stimulus, where no learning took place. The same rationale underlies the selection of the control for the ES. If the activity in response to this stimulus correlated with behavior but the activity to the other novel stimulus (to which responding was not extinguished) did not, we ranked these units as involved in extinction learning. In the reacquisition stage, we again used the other novel stimulus as control for the ES. With these controls we aimed at discarding units whose activity correlated with behavioral performance due to general changes in the firing patterns and not triggered by the learning process. Corrected for these unsystematic changes in firing rate we found a substantial number of neurons whose firing rate correlated significantly with behavioral performance specifically for the extinction stimulus (see table 4). We observed an obvious trend towards negative correlations in all analyzed periods, except reacquisition. This means that more neurons tend to decrease their firing during acquisition (when performance gets better) and to increase the firing during extinction (when performance drops) during presentation of the extinction stimulus. When comparing the number of neurons whose activity correlated with both novel stimuli (fifth row: overlap), we found that a fraction of units is involved in the learning process of both novel stimuli. Please note, that there is no overlap in regard of units which correlated with the performance of both novel stimuli in extinction and reacquisition, since we explicitly excluded them. Additionally, we saw that the sum
of neurons whose activity was correlated in each stage with behavior was larger than the number of neurons whose activity was correlated across all stages. This is in line with results described above, that the majority of neurons were stimulus selective in specific stages only. Also similar to the results above, we saw a trend towards less neurons correlating across stages (decrease from acquisition to reacquisition). However, again, we have to consider, that stages are of different length and therefore providing different strength to detect an effect. The smallest absolute correlation coefficient which reached significance level was $r=0.21$ in the acquisition stage. When we apply this as a criterion for significant correlations in the other stages and therefore correct for the differences in power, we found that results for different stages were more similar (46 and 29 units for extinction and reacquisition, respectively).

In summary, the distribution of correlation coefficients showed that a substantial number of neurons reflect the behavioral changes during learning in different learning stages. Like the results in the previous section, one can observe specialized subclusters of neurons, i.e. neurons that are involved during the acquisition of an operant response are not necessarily the same which are involved during extinction. To further investigate possible subclusters and their incidence in the present sample, we next categorized units based on their firing pattern during the learning stages.

Table 4: Absolute number of neurons whose activity correlated with behavior. Number of neurons, split up for learning stage, stimulus and direction of correlation is shown.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Session ES/NS1</th>
<th>Session NS2</th>
<th>Ac ES/NS1</th>
<th>Ac NS2</th>
<th>Ex ES/NS1</th>
<th>Ex NS2</th>
<th>Re ES/NS1</th>
<th>Re NS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12</td>
<td>8</td>
<td>22</td>
<td>16</td>
<td>14</td>
<td>7</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>16</td>
<td>12</td>
<td>33</td>
<td>15</td>
<td>20</td>
<td>12</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Sum</td>
<td>28</td>
<td>20</td>
<td>46</td>
<td>31</td>
<td>34</td>
<td>19</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Overlap</td>
<td>3</td>
<td>9</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>
Figure 18: Neuro-Behavioral Correlation. Bars represent frequency of correlation coefficients for all units (yellow), units with significant correlations (green) and units which correlated significantly with the behavior for the specific stimulus but not with the control (red). Columns show correlations for the different stimuli (left: extinction stimulus; right: second novel stimulus) while rows depict correlation distributions for different learning stages.

4.3.2.5.4. Unit classification

Based on different theoretical perspectives in regard to (extinction-) learning which imply different activity patterns of single neurons over the course of learning, we classified the activity patterns of the recorded neurons into four categories: First, if extinction constitutes the erasure of the previously learned association (or forgetting), one would expect, that the activity patterns seen during acquisition wane in
extinction. For example, firing rates could increase during acquisition and decrease during extinction (“forgetting neurons”). However, as described in the introduction, several lines of research point to the fact that the association is still functional after extinction and therefore neurons could represent the association throughout the extinction stage, i.e. increasing firing rates during acquisition and sustained high activity during the other stages (“acquisition remaining neurons”). Another possibility is that neurons represent the second inhibitory association (extinction neurons) and therefore increase their firing rates during extinction. Finally, neurons could track the conditions of the actual situation respective the decision of the animal. Then, one would expect changes in the firing pattern of neurons which closely track the behavioral performance of the animals (“behavior tracking neurons”). In this sample of NCL neurons, we found neurons of each category. In figure 19-22 one example unit of every category is shown. Figure 19: This neuron was classified as “acquisition remaining neuron”, since its firing rate increased during the time course of acquisition leaning (in close relation to behavioral performance) and held this level of activity till the end of the experimental session even though performance on the behavioral level dropped during extinction (black line). Crucially, the activity of the control condition (FS) did not follow the same dynamic. The neuron depicted in figure 20 was classified as “forgetting neuron”, because it showed increasing firing during acquisition like the former unit but decreased activity during extinction (like behavioral performance). Again, the control condition (green line, NS2) followed a different dynamic. The fact that this unit did not increase firing again during reacquisition distinguished it from a behavior tracking neuron like it is shown in figure 22 where firing increased during acquisition, decreased during extinction and increased again during reacquisition. The control condition (NS2) showed an increase at the end of the session, only. Finally, figure 21 depicts an example of an “extinction neuron” which selectively increases firing during extinction while control activity stayed low (green line, NS2). Interestingly, we observed different numbers of neurons in each category. Figure 23 shows the percentage of neurons which were classified based on their firing pattern during the three learning stages as “acquisition remaining”, “extinction”, “forgetting” or “behavior tracking neurons”. Only a small fraction of neurons displayed firing patterns which could be interpreted as “acquisition remaining” or “forgetting neurons” (5 units or 4% and 6 units 5% of all recorded neurons respectively). The majority of neurons which fall
in any of the categories were extinction neurons (18 units or 14%) while another substantial amount of units was classified as dynamic change neurons (10 or 8%). Because Do-Monte et al. (2015) recently reported that stimulating principal neurons in the IL during extinction (resembling the activity pattern of the here described “extinction neurons”) induced faster extinction, we examined how many neurons of the respective category were classified as inter- or principal neurons. While for “acquisition”, “forgetting” and “behavior tracking” neurons roughly 1/3 of the neurons were classified as interneurons, indeed only 17% (3 out of 17) of the “extinction neurons” were classified as interneurons. This means that the majority of “extinction neurons” were principal neurons.

Figure 19: Example “acquisition remaining neuron”. The firing rate of a neuron over the time course of one session (x-axis) and within one trial (y-axis) is shown. This neuron increased its firing rate during acquisition (until green line) and activity stayed high until the end of a session. Red line displays the mean firing rate within one trial for the ES. Black line depicts behavioral performance for the ES. Green line illustrates the control condition where the firing rate did not follow the same dynamic.
Figure 20: Example “forgetting neuron”. The activity of a neuron over the time course of one session (x-axis) and within one trial (y-axis) is shown. This neuron increased its firing rate during acquisition (until green line), decreased its activity during extinction (until yellow line) and further stayed low during reacquisition. Red line displays the mean firing rate within one trial for the ES. Black line depicts behavioral performance for the ES. Green line illustrates the control condition for which the firing rate did not follow the same dynamic.

Figure 21: Example “extinction neuron”. The chance of a neuron’s activity over the time course of one session (x-axis) and within one trial (y-axis) is shown. This neuron increased its firing rate specifically during extinction (between green and yellow line). Red line displays the mean firing rate within one trial for the ES. Black line depicts behavioral performance for the ES. Green line illustrates the control condition, which did not follow the same dynamic in firing rate.
Figure 22: Example “behavior tracking neuron”. The activity of a neuron over the time course of one session (x-axis) and within one trial (y-axis) is shown. This neuron increased its firing rate during acquisition (until green line), decreased its activity during extinction (until yellow line) and finally showed increased firing during reacquisition, again. Red line displays the mean firing rate within one trial for the ES. Black line depicts behavioral performance for the ES. Green line illustrates the control condition for which the firing rate did not follow the same dynamic.

Figure 23: Unit classification. Black bars show percentage of units, which were classified based on their firing rate across the session as “acquisition remaining”, “forgetting”, “extinction” or “behavior tracking” neurons. Red bars indicate number of units, which were classified as interneurons.

4.3.2.5.5. Interaction between neurons

The response pattern of “extinction” and “behavioral tracking neurons” resembled what has been described as coding pattern for the infralimbic (IL) and prelimbic (PL) cortex (see introduction or Lithik, 2008), respectively. Because histological analyses concerning electrode position are not finished yet, we cannot draw a final conclusion about possible anatomical clustering of neurons. However, we found 10 pairs of neurons with opposing activity patterns, which were simultaneously recorded. Next, we constructed cross-correlograms between simultaneously recorded units of different/ opposing categories to analyze potential direct interaction. Indeed, one pair showed signs of interaction in the cross-correlogram. This pair was already used to illustrate inhibition in figure 14E and were shown as “extinction”
and “behavior tracking” example neurons in figure 21 and 22. As particular detail it should be said that these two neurons were classified (based on their spike waveform as described above) as principal and interneuron, respectively. See top right corner of figure 14E for the respective waveforms. This implies that we do not see a clustering of neurons with opposing coding pattern like it can be shown for the PFC with the subdivision of infralimbic and prelimbic cortex.

The result concerning the interaction is of course of anecdotal nature and we were interested in the general interaction pattern of simultaneously recorded neurons and if we would find similar changes during learning or more opposing effects, rather. Thus, we correlated the activity of simultaneously recorded neurons during presentation of the extinction stimulus on a trial-by-trial basis. Figure 24 depicts the distribution of correlation coefficients for all possible pairs of simultaneously recorded units. Yellow bars show all possible pairs while green ones depict pairs with significant correlations. Roughly two third of the units pairs were correlated positively while one third exhibit a negative correlation. Because this proportion matches the one of putative principal and interneurons depicted in figure 14, we wondered if one type of neurons would be predominately responsible for one kind of correlation. However, we observed, that for both positive and negative correlation roughly in 50% of the cases interneurons are involved (red bars). One would expect more interneurons to be involved in opposing activity pattern, if they span from a direct interaction. This distribution implies that the negative correlation between the activity of neurons is probably also due to opposing input.

![Figure 24: Correlation of activity of simultaneously recorded neurons. Bars show frequency of correlation coefficients (Pearson's r) for all possible pairs of simultaneously recorded neurons. Color codes identify significant correlations (green, \( p < 0.05 \)) and correlations where one of the units was classified as an interneuron (red bars).](image)

Overall, the analysis of interaction pattern and classification showed that more neurons in the NCL showed changes in firing rate specific during extinction or tracked
the behavioral changes across the whole session. In addition, some of the units which were simultaneously recorded exhibited opposing dynamics during learning also showed interaction in the cross-correlogram which was in line with the distribution of correlations of all simultaneously recorded neurons, where positive as well as negative correlations can be observed. Thus, the neurons of this sample with specific/ opposing firing pattern show no anatomical clustering.

4.3.2.5.6. Selectivity between novel stimuli during the course of learning

In several studies investigating the mechanisms of learning on a single neuron level, increased stimulus selectivity for the to-be-learned stimuli was found to be a predictor for better behavioral performance (Assad et al, 1998). Because we observed a substantial proportion of neurons differentiating between the novel stimuli in the different learning stages, we also investigated the selectivity of these neurons across the stages. Thus, we computed a selectivity index based on the firing rates for the two novel stimuli for each trial (see material and methods) for the units which we before classified as differentiating between novel stimuli in any of the stages. Based on the results of the unit classification, we expected units to differentiate between the two novel stimuli also in extinction even though behavioral discrimination is worse in this stage. We assessed for every unit (which was differentiating in any of the stages (n=70)) the time point where the mean selectivity over ten trials was maximal and identified the learning stage in which this was the case. The percent of neurons which showed the maximum of selectivity in acquisition, extinction or reacquisition, respectively is shown in figure 25A. 42 % and 39% of the neurons differentiated maximally during acquisition and reacquisition, respectively. 19% of the units showed maximal selectivity during extinction. Example for units maximally differentiating during extinction are shown in figure 25BD (orange line, selectivity index) while C depicts a unit which differentiated maximally during acquisition and shows substantial differentiation also during reacquisition. Magenta and Cyan lines depict the firing rate across the recording session. These units were classified as an “extinction” (B, D) “behavior-tracking neurons” (C).
4.3.2.6. Reconstruction of recording sites

Recordings were completed for one bird (485) until now. Animals are highly trained (up to 7 month) and will participate in similar experiments in the future, as it is usual for highly trained non-human primates. However, surgery procedures were well established. The experimenter is a seasoned surgeon and in previous studies no bird had to be excluded from the sample due to incorrect electrode position. Figure 26 shows a coronal slice of the pigeon brain (stereotaxic atlas of Karten and Hodos (1967)), with the estimated position of the electrode track of pigeon 485 superimposed. It was placed within the borders of the NCL as defined by Herold et al. (2011).

Figure 26: Histological results. Coronal slice of the pigeon brain with estimated electrode position for subject 485. AP = Anterior-Posterior-Axis. Modified after Karten and Hodos (1967).
4.4. Discussion

The aim of the present study was to identify neuronal correlates of learning on a single neuron level with a focus on dynamic changes during extinction. Therefore, we designed a behavioral task for pigeons, which encompassed three learning stages (acquisition, extinction and reacquisition) in one experimental session and allowed for the simultaneous recording of single neurons’ activity. We could show that animals were able to repeatedly go through the task (with new stimuli each session) and acquire, extinguish and rapidly reacquire an operant response. With five animals performing this task, we were able to record the activity of 124 single units of the nidopallium caudolaterale, the avian functional equivalent of mammalian prefrontal cortex. We found that a substantial number of neurons changed their activity pattern in close relation to the changes seen on the behavioral level with different subpopulations being specifically involved during a particular learning stage. Additionally, we found four cluster of neurons with certain response profiles over the time course of learning. Among them, the cluster of so called “extinction neurons” (specifically active during the extinction stage) comprised the majority (14% of all recorded neurons). These are mainly principal neurons, resemble activity pattern observed in the IL of rodents during extinction and probably provide a teaching signal for behavioral inhibition. Another 8% of the neurons closely tracked behavioral changes throughout the experimental session (“behavior tracking neurons”). These neurons resemble activity changes seen in the PL of rodents and we hypothesize them to be involved in the selection of an appropriate response under changing reward contingencies. These results emphasize the role of prefrontal areas in the selection of appropriate actions and inhibition of no longer appropriate ones.

4.4.1. Behavioral task

We designed a novel behavioral task that allowed us to record the activity of the same single units across three stages of learning since acquisition, extinction and reacquisition were realized in one experimental session lasting approximately 3h on average. Animals repeatedly performed this task and performance level increased, decreased and rapidly increased again during acquisition, extinction and reacquisition, respectively (see figure 12AB).
The number of trials animals needed to fulfill criteria for acquisition (> 85% correct choices), extinction (< 65% correct choices) and reacquisition (> 85% correct choices) indicated that animals took longest to initially acquire the correct response. This is in line with the experimental observation that acquisition takes more time than (rapid) reacquisition. However, two issues have to be considered: first, animals had to learn the correct response for two stimuli simultaneously during acquisition while during extinction behavior changes were expected for one stimulus only. In addition, performance for novel stimuli did not always begin at chance level, leading to differences in the extent of behavioral change that had to be executed in order to fulfill the respective criteria of the learning stages. Some animals exhibited a side bias leading for example to sub-chance performance (10% correct) for one stimulus at the beginning of the session (see magenta line in figure 12A). To reach criterion the animal in this example had to improve performance by 75%, which is obviously higher than a decrease during extinction of 20% (85-65%). Even if performance started at 50% chance level, the difference would still be higher. Therefore, the interpretation of differences in the absolute length of acquisition in comparison to the extinction stage is complicated. However, the to-be-overcome difference in performance between the extinction and reacquisition stage is equal (85 % down to 65% (extinction) and 65% up to 85% (reacquisition)) and one can conclude that reacquisition is more rapid than extinction. Additionally, differences in the amount of trials between acquisition and reacquisition are so tremendous (four-fold decrease from acquisition to reacquisition) that it is very unlikely that this difference solely results from the conditions described above but also reflects rapid reacquisition, i.e. a faster increase in responding (performance) during reacquisition than during first acquisition. As described in the introduction, faster reacquisition constitutes one reason, why it is assumed that extinction does not reflect forgetting or the erasure of the acquisition memory. During reacquisition the organism can built upon the previously learned association and performance increases rapidly.

To investigate other signatures of extinction on the behavioral level besides decrease in performance (percent correct responses), we looked for changes in pecking and reaction times regarding the extinction stimulus (ES). We observed a clear decrease in the number of pecks in response to the ES during extinction. However, responses stayed attenuated during reacquisition and therefore this measurement of
associative strength did not show rapid reacquisition; probably because high pecking rates are not conditional on the outcome. In fact, animals only had to emit at least one peck. In regard to reaction times, we saw an increase over the time course of the session for all stimuli. Therefore, this increase most likely reflects loss of motivation due to saturation and fatigue at the end of a long-lasting experimental session.

In conclusion, behavioral results indicate that the experimental design is suitable for investigating the neuronal correlates of dynamic behavioral changes during learning, as animals were successfully performing this task, i.e. flexibly adapting their behavior to repeatedly changing reinforcement conditions and stable performance for familiar stimuli with stable reinforcement conditions. Concerning the number of performed trials as well as the ability to flexible adapt to changing conditions within one experimental session they are on par with non-human primates.

4.4.2. Electrophysiological recordings

4.4.2.1. Methodological improvements

The first aim within my PhD thesis regarding electrophysiological recordings was to improve signal quality, i.e. reducing artifacts and to increase the yield of each recording session in terms of the number of simultaneously recorded neurons. We doubled the number of electrode wires, reconstructed the microarray, made use of a different plug (tightly but reversible connected to the headstage) and gold-plated electrode tips. This resulted in a three-fold increase of the average number of neurons recorded per experimental session when compared to a previous study. With the doubling of the electrode wires, one would expect twice the number of neurons in each session. However, the number was even higher, indicating an additional improvement by the gold-plating protocol and the new connector. This improvement was of particular importance for the success of the study for two reasons: first, animals were trained extensively on this task until they could undergo surgery and neuronal activity could be recorded. This increased the value and importance of single animals successfully performing the task. Thus, a high yield in terms of neurons in every successful recording session helped to reduce animal use and assured the general success of the investigation. Furthermore, only the higher number of
simultaneously recorded neurons allow for a systematic investigation of interaction patterns between neurons.

Another result of the gold plating protocol was probably the higher number of thin-spiking neurons (as classified by a k-means algorithm), which probably resemble type II neurons in Kröner et al. (2002) and inhibitory interneurons in mammalian cortex (McCormick et al., 1985). Because of the reduced background noise when recording with gold-plated electrode tips, the spikes of interneurons with smaller amplitudes on average (see figure 14C) were more likely to be detected in this study. In turn, the higher incidence of this unit class explained the higher mean firing rate in the present study compared to previous ones (Starosta et al., 2013).

4.4.2.2. Classification of neurons into putative projecting- and interneurons

Based on the waveform width of the action potentials (mean full width at half maximum for the first and second peak), we classified all recorded units into two clusters: a larger cluster of broad spiking neurons with an on average lower firing rate compared to the second smaller cluster of thin-spiking neurons (mean: 1.9 vs. 7.4 Hz). We believe that these two cluster correspond to the two general neuron classes observed in mammalian cortex: principal- or projection- and inter-neurons. Of course, we cannot say for sure, if the smaller cluster of neurons indeed comprises a population of interneurons since anatomical information about the clusters is not available. However, an anatomical study (Kröner et al., 2002) investigating the morphological and physiological properties of NCL neurons in chicks indicates that waveform width in combination with firing rate is a good predictor for the axon length of neurons and consequently for their group identity (as projection neurons develop longer axons than inter neurons). The relative size of the identified subgroups (12 vs. 77%) roughly corresponds to the one we observed in this data set (30 vs. 70%). Furthermore, we observed interaction pattern suggestive of an inhibitory or excitatory connection between simultaneously recorded neurons (figure 14DE). One could argue, that the time range of the observed interaction is very small and physiologically unrealistic, because the excitatory interaction in figure 14F occurs within the first millisecond. However, there are studies indicating that the influence of the firing of one neuron on the firing probability of another one can become effective within very short time scales (Moore et al., 1970). Finally, in mammals it is a common approach to classify neurons based on their waveform into two cluster
and recent optogenetic targeting of interneurons gives further support for this clas-
sification method as it resembles the results of classification by optogenetic target-
ing (Kepecs and Fishell, 2014). In summary, we are confident to say that the rec-
orded neurons can be classified as thin-spiking interneurons and broad-spiking-pro-
jection-neurons.

4.4.2.3. Functional properties of NCL neurons

We found that 44 out of the 124 recorded units exhibited significant motor- modu-
lation, i.e. the distribution of firing rates closely around the time point of pecking
(±100ms) did not follow a flat (expected) distribution. The relative number of neu-
rons showing this modulation was comparable to previous reports in freely moving
animals (27% & 41% in Lengersdorf et al.(2014a); Starosta et al. (2013) respec-
tively. However, it was higher than the number (4%) in a head-fixed preparation
(Kalt et al., 1999), which is probably due to a general higher motoric activity in
freely moving compared to head-fixed birds.

A substantial number of neurons (93 or 75%) decreased (35 or 28%) or increased
(58 or 48%) their firing rate compared to baseline during the sample phase (stimulus
presentation). This may be caused by the visual input due to the presented stimuli
as well as motor-actions as animals were pecking during stimulus presentation. Fur-
thermore, approximately half of the recorded neurons (70) were stimulus-modu-
lated, meaning that the response to at least one of the stimuli was significantly dif-
ferent to one of the other stimuli. Since motoric responses for the stimuli were
roughly the same for all stimuli (figure 12CD), this modulation is probably due to
visual input (stimuli identity) as well as the meaning of the stimulus as for example
associated outcome, novelty or required response in the following phase (choice)
of the trial. Indeed, we observed that 46% of the recorded neurons differentiated
between novel and familiar cues (see figure 16C for an example). A response pat-
tern which was also reported for prefrontal neurons in the macaque (Asaad et al.,
1998). Again 46% of the units differentiated between stimuli, which were associ-
ated with a left or right choice response. Thus, one could predict the upcoming
choice of the animal based on the firing rate of neurons during stimulus presenta-
tion. This was reported previously for NCL as well as prefrontal neurons
(Lengersdorf et al., 2014a; Kim and Shadlen, 1999). Finally, 26 of all recorded units
differentiated between novel and familiar stimuli and additionally between stimuli
associated with a left or right peck, converging signals for the correct response and the novelty of the cues. The reported differentiation pattern suggest a general coding scheme in NCL neurons for the selected action and novelty of presented stimuli which they share with PFC neurons and provides further support for assumption of a functional comparability of NCL and PFC (Güntürkün, 2005b; Güntürkün, 2012; Kirsch et al., 2008).

4.4.2.4. Differentiation between novel stimuli

Since acquiring the correct responses to novel stimuli was the major challenge in our paradigm, we investigated how many neurons differentiated between the two novel stimuli during stimulus presentation. We found that 70 units (56%) differentiated between the novel stimuli (NS) in at least one of the learning stages, which can be interpreted as coding for the associated choice response required later in the trial. This is similar to coding left and right responses as described earlier since the specific novel stimuli are uniquely associated with a left or right peck response.

As we were interested in the coding pattern of single neurons across different learning stages, we looked at the number of neurons differentiating between NS split up for learning stages. We realized that roughly one third of the differentiating neurons, exhibit a distinct firing pattern to one novel stimulus compared to the other in each stage (figure 17B). This pattern could be the result of several coding pattern of neurons across learning stages: firstly, the same thirty percent of neurons could be differentiating in all three stages. Secondly, it could be that neurons show differential responding in one stage exclusively or, thirdly, there may exist a combination of both. To examine, what applies for the present sample of units, we divided the subpopulation of neurons differentiating in one stage, based on their coding pattern in the other stages. This analysis revealed that the majority of neurons (34 or 56%) showed differential firing in one learning stage only, indicating a specialization of subpopulations for particular learning stages. Finally, we examined which stage neurons were specialized for and found that 38, 21 & 41 % of the neurons were specialized in coding in the acquisition, extinction and reacquisition stage, respectively. Thus, less neurons differentiated only in the extinction stage and neurons more often differentiate between the novel cues when they were clearly associated with opposing responses. However, that some neurons differentiated best during
extinction at all, indicates extinction related processes are coded alongside the selected action.

In conclusion, the observed formation of subgroups indicates that two different processes may underlie the differentiation between the novel stimuli. During acquisition and reacquisition the differentiation could be used as a signal to select the appropriate response; meanwhile, differentiation during extinction could be interpreted as a signal due to differences in the learning process currently occurring for the given stimulus. It cannot reflect the response selection like in acquisition or reacquisition, because responses become more similar during extinction (executing a choice to the other side), while neuronal differentiation gets stronger. An alternative explanation would be the coding of a violated (reward) expectation triggering behavioral inhibition and consequently extinction. I will expand upon this topic later in the discussion of the response profile of single neurons as well as in the general discussion.

4.4.2.5. Selectivity across learning

Studies conducting single unit recordings during the time course of learning often use the selectivity of firing rates between different stimuli as an index for learning. Thereby, a selectivity index is computed which reflects the difference of firing rates in two conditions divided by the sum of the firing rates. Asaad et al. (1998) for instance could show that selectivity in prefrontal neurons’ responses for different stimuli increased over the time course of learning and occurred successively earlier in a trial. Similarly, we found that neurons exhibit different degrees of selectivity across the experimental session. However, the focus of the present study laid in the comparison between learning stages. Accordingly, we assessed the time within the recording session where units exhibited maximal selectivity between the two novel stimuli. We found that most neurons showed the highest selectivity for one of the novel stimuli during the acquisition or reacquisition stage, indicative of coding for the correct response to be executed. Interestingly, we also found a substantial fraction of neurons (13 or 19%) which exhibited the highest selectivity during extinction. If one assumes that the degree of selectivity predicts the discrimination performance or the upcoming response on a behavioral level, this result is unexpected. During extinction, the differentiation on the behavioral level, i.e. which response (left or right) is executed deteriorated, because animals chose the wrong side more
often. However, a subpopulation of neurons in the NCL differentiated best during the extinction stage. This result is similar to the one described above, that a subpopulation of neurons differentiated between the NS in the extinction stage only. Indeed the analyses are closely entangled, as the variables “how many neurons differentiate in specific stages” and “when do neurons differentiate best” are dependent on each other. Again, a possible explanation for the observed data pattern would be the coding of single neurons in the NCL for (reward) expectancy violation or behavioral inhibition.

4.4.2.6. Correlation between performance and neuronal responses during learning

To identify the relationship between the neuronal activity and behavioral performance on a finer time scale, we correlated these two variables. In detail, we correlated the mean activity during sample presentation with behavioral performance (percent correct). Correlations were computed across all learning stages as well as for specific ones. As described in the results section in more detailed, we controlled for unsystematic changes in the firing rate in time by excluding neurons whose activity correlated with the performance concerning another stimulus, where no acquisition or extinction learning took place. Corrected for this, we found a substantial amount of neurons whose activity correlated with behavioral performance changes. This was true for the time course of the complete session as well as for specific learning stages. Strikingly, we observed again, that some units are specifically involved during certain learning stages, because the sum of the number of units correlated in each specific stage outnumbered the number of units correlating with behavior over the whole session. This is in line with previous reports investigating neuronal activity during learning that different subpopulations are involved in acquisition and extinction (An et al., 2012; Herry et al., 2008).

Overall, we noticed a trend towards more negative correlation meaning that neurons more often increased their firing when performance dropped and decreased it when performance improved. This effect was more pronounced for the extinction stimulus (ES) in the acquisition and extinction stage where twice neurons correlated negatively than positively with performance. A similar pattern was observed in our previous study (Starosta et al., 2013) where single unit activity more often correlated negatively with behavior in a generalization task. As above, we interpret this
result as a potential signature of the violation of reward expectation and the behavioral inhibition in response to non-reward-predicting stimuli. In addition, we observed quite a few cells, that were engaged in the acquisition for both novel stimuli (fifth row: overlap). Most units’ activity was correlated with improvements in behavioral performance for a specific stimulus. This appears to be a general phenomenon when investigating the neuronal correlates of learning: Wirth et al., 2003 also describe learning related changes in the activity of single hippocampal units in response to specific stimuli while no changes in response to other stimuli were observed.

4.4.2.7. Unit classification

Based on the correlation described above, we classified units according to their response pattern throughout the learning stages. Considering possible neuronal signatures of acquisition and extinction memory, let us assume the following categories: 1) forgetting: one assumption about extinction is that the observed decrease in responding is due to forgetting and consequently the erasure of the memory trace. If this assumption hold, we should find coding pattern of neurons, which increases with the acquisition of an association and wanes during extinction. Indeed we found that six neurons exhibited such coding pattern, namely increase in firing rate during acquisition (positive correlation) as well as decreasing activity during extinction (positive correlation). 2) acquisition remaining: As extensively discussed, experimental data suggests that during extinction, the first memory remains functional. A possible neuronal correlate for this could be the increase in firing during acquisition (positive correlation) and stable elevated firing throughout the remaining session (no further correlation with behavior). We recorded five neurons (4%) with such an activity profile and termed them “acquisition remaining neurons”. Furthermore, theoretical assumption also predicts a second inhibitory connection between the stimulus, response and outcome during extinction and we were curious to find a neuronal correlate of this. We found that 14% of neurons (18 out of 124) selectively increased their firing during extinction (“extinction neurons”). Finally, we expected neurons closely tracking the changes in reward contingencies and consequently performance, as prefrontal regions are associated with action selection and reward pro-
cessing (Lengersdorf et al., 2014a; Koenen et al., 2013). Ten neurons (8%) displayed close tracking of behavioral changes in form of a positive correlation throughout the session (“behavior tracking neurons”).

In the following, the four categories of response profiles are discussed in terms of their potential function during learning. Neurons classified as “forgetting”, “acquisition remaining” as well as “behavioral tracking” neurons increased their firing rate during acquisition. This increase could be interpreted as a signal of the developing association between the stimulus and the appropriate response to obtain a reward. Closely related to that is the increase in reward probability during acquisition (because the animals more often chose the correct response) which could be reflected in the elevated firing rates. Such an involvement of single neurons in the coding of an acquired memory has been reported to a similar degree for the hippocampus (Wirth et al., 2003) the amygdala (An et al., 2012; Herry et al., 2008; Quirk et al., 1995) as well as for the prefrontal cortex (Asaad et al., 1998) including PL (Burgos-Robles et al., 2009). A number of studies show the reflection of reward expectancy of single neurons of the PFC (Kennerley and Walton, 2011). Additionally, coding for an acquired memory (Kirsch et al., 2009; Kirsch and Güntürkün, 2005) as well as reward expectancy (Kalenscher et al., 2005; Starosta et al., 2013) has been reported for the NCL as well. However, the studies of Wirth, Asaad and Kirsch for example could not differentiate between the three categories outlined above as they stopped recording after acquisition or solely reversed the stimulus-response assignment. By continuing recording during the extinction stage, we were able to separate “forgetting” and “behavior tracking” neurons from “acquisition remaining” neurons, as the first two decreased their firing during extinction while firing remained elevated for the last mentioned. Therefore, response profiles for “behavior tracking” and “forgetting” neurons reflect what An et al. (2012) described as “extinction susceptible” for neurons that decrease their firing during extinction. In addition, the elevated high firing in extinction of “acquisition remaining” neurons was also observed by An et al. (2012) and termed extinction resistant. Similarly, Herry et al. (2008) and Quirk et al. (1995) report single units in the amygdala whose activity correlated positively with the expression of fear during acquisition. However, the absolute amount of these neurons was quite low in our sample, indicating that the association is stored probably somewhere else. Finally, we were able to disentangle “forgetting” and “behavior tracking” neurons depending on their
response profile during reacquisition. Again, comparable to what An and Herry described for reacquisition and renewal, some of the units renewed their response during reacquisition while the activity of a smaller subgroup stayed attenuated. This is in line with previous reports that extinction involves to some extent also the erasure of the memory (Quirk et al., 2010) as the cluster of “forgetting neurons” imply. The fourth activity profile we observed was elevated firing during and restricted to the extinction stage (“extinction neurons”), which was again also described as “extinction neurons” by Herry et al., 2008 for the amygdala. This change in activity could be interpreted as a signal for violated reward expectation and inhibition of previous behavior during extinction. Similarly, in an investigation of coding properties of NCL neurons (Starosta et al., 2013), we also observed coding for events associated with non-reward, providing further support for the role of the NCL in inhibition of non-reinforced actions. Furthermore, a study conducting single unit recordings in the PFC reported elevated activity of single neurons related to extinction memory (Milad and Quirk, 2002). This elevated activity was observed during extinction recall only, however, which was not tested in the present study in a comparable way. Finally, the activity pattern of “extinction neurons” is analogous to reports of elevated firing of single unit in the infralimbic cortex as well as elevated activity in the human homologue (ventromedial prefrontal cortex) during extinction training (Phelps et al., 2004; Holmes et al., 2012; Chang et al., 2010; Milad and Quirk, 2002; Lissek et al., 2015). This emphasizes the role of the prefrontal cortex for inhibiting inappropriate actions during extinction learning and recall. Finally, the elevated firing during extinction could also be interpreted as a correlate of increased attention as prefrontal regions were shown to be involved in attention processing (Visintin et al., 2014; Rose et al., 2010).

4.4.3. “Extinction neurons” – expectancy violation, attention or behavioral inhibition?

We observed two main activity pattern in NCL neurons during learning and called the respective neurons “extinction neurons” and “behavior tracking neurons”. While the pattern exhibited by “behavioral tracking neurons”, i.e. correlation with behavioral performance in combination with reward probability, has been described for the NCL extensively (Kalenscher et al., 2005; Koenen et al., 2013; Starosta et al., 2013), we are the first ones reporting a selective increase in activity over time.
when a response, maintained by appetitive outcomes, is no longer reinforced. Above it was mentioned, that the increase could signal the violation of the reward expectation during extinction. However, because our paradigm allows for the investigation of the temporal development of this signal, we exclude this possibility for the following reason: the violation of the reward expectancy is highest at the beginning of the extinction stage, because the reward is predicted to 100% and then omitted. The repeated omission of the reward decreases the expectancy and thus the extent of violation should decrease over the time course of extinction (learning), too. A similar assumption can be made for attentional processes as they are assumed to be modulated by expectancy violation. However, the signal we observed in “extinction” neurons show exactly the opposite dynamic. It is negatively correlated with behavioral performance, i.e. increasing over time and is highest at the end of extinction (see figure 21 for an example). This does not fit with the coding of the expectancy violation but is in line with the interpretation of the signal for triggering behavioral inhibition. As the former correct response is executed decreasingly over the time course of extinction the activity of the “extinction neurons” is increasing.

In summary, we observed response profiles of neurons, which are in line with all four theoretical assumptions about learning related signals in the brain. However, the majority of neurons showed a selective increase during the extinction stage (“extinction neurons”) which resemble response pattern seen in IL and probably provide a signal to trigger behavioral inhibition of formerly correct responses. On the other hand, a substantial subgroup of neurons closely track reward probabilities and behavioral changes in performance across the experimental session (alike PL neurons). Both is perfectly in line with the assumed function of prefrontal areas in selecting appropriate actions and inhibiting inappropriate ones in changing environments.

4.4.3.1. Anatomical differentiation

As suggested above, the described response profiles of “behavior tracking” and “extinction neurons” resemble those classically observed in the prelimbic and infralimbic cortex, respectively. While high activity in the prelimbic cortex during acquisition and retrieval is thought to trigger the expression of conditioned fear, elevated activity in the infralimbic cortex is assumed to suppress conditioned fear
during and after extinction (and a similar pattern is suggested for drug-seeking behavior (Millan et al., 2011)). It should be noted that, there exists no subdivision of the NCL as it was described in mammals (Genovesio et al., 2014). Since histological analyses concerning electrode position are not finished yet, we cannot draw a final conclusion about possible anatomical clustering of neurons. However, we recorded opposing response profiles simultaneously in the same experimental session and found negative as well as positive correlations between the firing rates of simultaneously recorded neurons. This means that neurons with opposing response profiles were closely intermingled in the NCL. This resembles a distribution, which was described for the amygdala by Herry et al. (2008), rather than for the prefrontal cortex. Nevertheless, strong interaction between PL and IL was also described (Sotres-Bayon and Quirk, 2010) in a similar way like we do, seen the interaction pattern seen in the cross-correlograms (figure 14) and the overall correlation between simultaneously recorded neurons (figure 24). It is reasonable, that neurons with opposing response profiles interact with each other directly or receive different input as well as project to different down-stream structures to produce in the end-adapted behavior.

Interestingly, the most similar response profile of single units to what we describe here were found in single units of the amygdala. Therefore, we closely compared the activity changes with what was described by Herry et al. (2008) and An et al. (2012). Herry argued, that the two subpopulations of neurons he described allow for a rapid shift between high and low fear stages. Because prefrontal regions are highly interconnected with the amygdala in rodents and pigeons (Peters et al., 2009; Kröner et al., 2002), similar signals are expected. For the expression of fear, the projection from the PFC to the amygdala has also been shown to drive or inhibit fear expression (Sotres-Bayon and Quirk, 2010). It is reasonable, that also in the appetitive domain, prefrontal region send a signal to the amygdala to drive certain behavioral states or inhibit those. Actually, in the case of behavioral inhibition it is more likely that the signal in the amygdala is the mirror image of what is seen in prefrontal regions than the other way around, because of their assumed different roles concerning the storage of memory (amygdala) vs. the control of behavior (PFC).
In conclusion, all collected and analyzed measurements (correlation, selectivity and classification) point to the fact, that coding properties of NCL neurons reflect two main objectives: Firstly, the upcoming choice of the animal so that activity changes are closely tracking behavioral performance/ expected reward and, secondly, a teaching signal for downstream structures triggering behavioral inhibition. While the coding for an upcoming response as well as reward expectancy in NCL neurons have been shown before (Kalenscher et al., 2005; Lengersdorf et al., 2014a), the present investigation is the first to describe a single neuron correlate of extinction learning of an appetitive response in a frontal brain region. We believe, that this signal could act as a teaching signal for association storage and inhibiting of previous motor plans in down-stream structures (for instance to the amygdala or striatum).
5. General discussion

In this thesis, I investigated behavioral and neuronal mechanisms of extinction learning. The first two studies were behavioral experiments and focused on the context-specificity of acquisition and extinction memory. In the third study, I investigated responses of single neurons in the nidopallium caudolaterale (NCL), the functional equivalent of mammalian prefrontal cortex, during acquisition, extinction and reacquisition of an operant response. A detailed discussion of the results is given in the respective chapters, so that I will here summarize the results and their implication as well as combine the insights from the behavioral and electrophysiological data of the three studies. Finally, I will discuss potential shortcomings in combination with open questions and how they could be addressed in future research attempts.

In the behavioral studies of this thesis, we found that, both acquisition and extinction memory is context-specific, if the conditioned response is acquired and extinguished (in response to different stimuli) under the same conditions (i.e. simultaneously). This contrasts with classical assumptions of the mechanisms of context-specificity which states that an extinguished response is more context-specific, because it displays the second learned information about a stimulus as well as an inhibitory association between stimulus and outcome (Bouton, 2004). Our data shows that a first learned information about a stimulus, which is at the same time an excitatory association, is stored and expressed in a context-specific way. This is in line with the attentional theory of context processing (ATCP, Rosas et al., 2006) proposing that, whether a learned association is stored in a context-specific way or not, depends on the circumstances experienced while it was learned, namely the attention paid to the context and its experienced relevance.

On the neuronal level, we discovered that the activity of a substantial subgroup of single units in the NCL correlated closely with behavioral performance, i.e. activity changes across learning stages tracked learning-related changes on the behavioral level. In addition, the changes on the neuronal level led to differences in the degree neurons differentiated between the two novel stimuli (NS) to which responding was acquired or extinguished. While the majority of neurons differentiated best during acquisition or reacquisition, in a substantial subgroup of neurons firing rates for NS
were maximally distinct during extinction. We interpreted this as a neuronal correlate of two functions dedicated to prefrontal areas: action selection and behavioral inhibition. During acquisition and reacquisition, the NS have to be differentiated to decide which action to execute in the following phase of the trial (left or right peck) to in order to obtain a food reward. In contrast, during extinction, the formerly correct action (a left peck, for example) is inhibited and performance declines, because the other option is chosen more frequently (right peck). We think that this increased differentiation reflects the contrast between the stimuli during extinction in the sense that a former response has to be inhibited only for one of the NS. The results of the classification of neurons based on their activity pattern across the session point in a similar direction. The neurons of the two major subpopulations we observed, either closely tracked the behavioral changes (“behavior tracking neurons”) or exhibited elevated activity during extinction learning (“extinction neurons”). We interpret these signals as reflecting reward expectancy associated with the stimulus and triggering action selection in case of the first population. For the second, we believe, that the activity realizes a signal for behavioral inhibition during extinction. Together, the results concerning selectivity/differentiation of firing rates as well as classification emphasize the role of the prefrontal cortex in action selection and behavioral inhibition as it was shown for the prelimbic and infralimbic cortex in rodents.

5.1. Combination of behavioral and neuronal insights - a special role for prefrontal areas in extinction

In the introduction, we argued that the PFC plays a major role in extinction learning because of its integrative function for several lines of information. This is in line with the presented results here, where single neurons in the avian equivalent of the PFC exhibit activation pattern interpreted as signals for reward expectancy and behavioral inhibition in parallel. Even though, the PFC has been implicated in the process of extinction learning before and a very recent study using optogenetics showed that silencing of neurons in the infralimbic cortex blocks extinction retrieval (Do-Monte et al., 2015), we report a neuronal correlate in a prefrontal analog structure for the assumed inhibition associated with extinction learning in the appetitive domain for the first time.
We think that the elevated activity of “extinction neurons” could provide a teaching signal for down-stream structures like the amygdala or the striatum to store the CS-noUS association or inhibit the previously shown response. The PFC (or the analogue avian structure) is recruited when situation becomes ambiguous and previous actions have to be inhibited.

How could the assumed neuronal mechanism inspire the theories in regard to mechanisms of context-specificity? It has been shown, that during extinction the hippocampus is gating amygdala signals to the PFC (Sotres-Bayon et al., 2012). This means, that the hippocampus modulates or sends information to the PFC, which in the end are important to decide which behavior is expressed. For example, the hippocampus could provide contextual information, i.e. where a stimulus is reinforced and where not. It is reasonable, that this gating also applies to stimuli for which no information about the context relevance is available, like it was for the newly introduced stimuli in phase two of the behavioral experiments. While the gating is present, this information is integrated into the memory for other associations (stimuli for which responding is acquired) as well. This could be of great importance during appetitive conditioning, since it has been shown that information about aversive events is generalized much more broadly than information about appetitive events (Schechtman et al., 2010).

5.2. Comparative remarks

As the presented studies were conducted using pigeons as the experimental animals of choice, the results presented here have comparative implications in addition to the behavioral and neuronal insights regarding an evolutionary highly conserved ability – learning. Within the scope of the behavioral experiments, we can show that pigeons are able to use context as cues to solve tasks and similar results have been reported before (Lengersdorf et al., 2014b; Rescorla, 2008). In addition, we saw that they clearly differentiate between stimuli for which the context was signaled as relevant or irrelevant, because they exhibited context-specific responding to novel CS but not for control stimuli, like human participants (Lucke et al., 2013; Lucke et al., 2014)

Finally, the electrophysiological recordings indicate like other several studies before (Lissek and Güntürkün, 2003; Lengersdorf et al., 2014b), that also the NCL
similar to the PFC plays a major role in the extinction of previously reinforced behavior. The combination of demonstrating similar signals in the pigeon brain as in mammalian brains with entirely new insights due to the sophisticated paradigm, strongly speaks for the usage of pigeons for the investigation of the neuronal mechanism of learning and memory (Güntürkün et al., 2014; Colombo and Scarf, 2012). It furthermore implies that insight about neuronal mechanisms of learning gained from the pigeon brain, can be transferred to other organisms like humans. It should be emphasized that we found exactly the same response pattern like the ones described during fear conditioning in the rodent PFC, even though, we recorded activity in a non-homologous structure and in an appetitive paradigm. This strongly supports a general mechanism for (extinction-) learning implemented in forebrain areas.

5.3. Potential shortcomings and outlook

In the behavioral studies, the effects of context-specificity for acquired responses are very strong and could be replicated in two studies for different experimental groups and various stimuli. Unfortunately, the insight into the underlying mechanisms is limited. Since animals did not show context-specific responding for the control stimuli, we can conclude, that the experienced context-relevance is critical. However, regarding the second proposed mechanism for inducing context-specificity, which deals with attention and experienced ambiguity, we are not able to draw any conclusion. In future experiments, one could again use latent inhibition as an indirect maker for attention. One should introduce a stimulus in a similar way in the acquisition stage as we did during extinction and test for latent inhibition later on. If animals pay more attention to the context during extinction, latent inhibition for the stimulus presented during this experimental stage should be more pronounced as for the stimulus presented during acquisition. This would allow for conclusion about the involvement of attention in context-specific learning. On the other hand, one could manipulate the expectancy violation using different compound stimuli, like we tried with reward amount in study one.

One possible shortcoming of the presented electrophysiological study is that is does not allow for insights into the long-term storage of memory. Since all three stages are realized within one experimental session possible neuronal correlates of the long-term storage could not be investigated. It has been described that extinction
training directly after conditioning leads to a deficit in long term storage of the extinction memory (Chang and Maren, 2009); an effect that is probably based on elevated firing of single units in the prelimbic cortex (Chang et al., 2010). This could have affected our data. However, the authors of these studies finally show, that this deficit in extinction is most likely due to stress effects that persist in the extinction stage and let brain region store extinction memory suboptimal (Maren, 2014). Our paradigm is appetitive in nature. Thus, we do not expect such an effect, when the extinction training takes place immediately after acquisition. However, it could be interesting to test, if an immediate extinction effect can be observed in the appetitive domain as well. It remains the constraint that the long-term-storage cannot be investigated, because reacquisition follows immediately after extinction. This critique is definitely true, but the focus of the present study was not the investigation of the long-term storage of extinction memory. Rather, we were interested in the neuronal signals associated with extinction and their dynamic changes while a response is extinguished. For this purpose, the chosen behavioral paradigm worked ideally.

Studies in non-human-primates (Puig et al., 2014) as well as in pigeons (Rose et al., 2013) showed an effect on learning new association when dopaminergic receptors were manipulated. Thus, it could be a next step to manipulate dopaminergic input to or metabolism in the NCL to see if the dynamic changes observed on the neuronal and behavioral level wane to a comparable degree when dopaminergic signaling is altered. As dopaminergic signaling was elucidated as an essential mechanisms in the formation of memory, it is very likely and first optogenetic studies suggest so (Steinberg et al., 2013), that dopaminergic signaling is also crucial for extinction learning. In a similar fashion, recent unpublished experiments from our lab show, that NDMR receptors in the NCL influence extinction as their blockade leads to a attenuation of extinction learning (Lengersdorf et al., 2015, submitted). Therefore, the combination of NMDR receptor antagonists with electrophysiological recordings could be a future direction to investigate causal relationships between neuronal activity and behavior.

Unlike most recent studies in the fear conditioning field (Courtin et al., 2014; Senn et al., 2014; Wolff et al., 2014; Do-Monte et al., 2015), we cannot draw causal conclusion about possible connection pattern or specific neuronal subtypes involved in the process of extinction learning. However, we observed that more neurons than
expected in the subpopulation of “extinction neurons” were principal neurons. Similar to what Do-Monte et al. (2015) described. Besides methodological issues in the combination of recent technologies and sophisticated behavioral paradigms, the problem arises due to the limited knowledge about the mechanism and firing patterns of single units in appetitive designs. As pointed out in the introduction, much less is known about single unit firing pattern in appetitive extinction procedures. As an example, a recent study point into the direction that the classic distinction between IL and PL cortex and their involvement in fear extinction and acquisition could hold in the appetitive domain as well (Sparta et al., 2014). However, we are just beginning to discover the similarities and differences of appetitive and fear extinction. Herry described seven years ago, “extinction neurons” in the amygdala and their specific connectivity was defined only recently. Description of neuronal firing pattern in appetitive (extinction)-learning as we can describe in our study, now can built the backbone for further investigations of the connectivity or causal involvement of specific neuronal subpopulations.

5.4. Conclusions

Learning that events in the environment can predict other critical ones is as important for survival as it is to learn that no relationship between two events exists anymore. These two learning processes are described by the terms acquisition and extinction, respectively. While the first two studies of this thesis investigated the behavioral mechanisms of context-specificity of acquisition and extinction learning, the last study focused on the neuronal signatures of these learning processes in the avian equivalent of the prefrontal cortex. Behavioral results point out, that the mechanism that induces context-specificity of extinction memory can be relevant for acquisition memory also, when both learning processes take place under the same conditions. Therefore, one can conclude, that acquisition and extinction share a common mechanism concerning the formation of context-specificity on the behavioral level. Additionally, single units in the avian forebrain exhibit response pattern that can be interpreted as a teaching signal associated with inhibition of previously optimal behavior similar to signals in the rodent infralimbic cortex. We conclude, that this structure is especially involved in the process of learning that a once predictive stimulus is no longer predictive of another salient event – extinction.
6. References


Haubrich J, Crestani AP, Cassini LF, Santana F, Sierra RO, Alvares LO, Quillfeldt JA (2015) Reconsolidation allows fear memory to be updated to a less aversive level through the incorporation of appetitive information. Neuropsychopharmacology 40:315-326.


Howard MW, Eichenbaum H (2014) Time and space in the hippocampus. Brain Res.


Lengersdorf D, Stüttgen MC, Uengoer M, Güntürkün O (2014b) Transient inactivation of the pigeon hippocampus or the nidopallium caudolaterale during extinction learning impairs extinction retrieval in an appetitive conditioning paradigm. Behav Brain Res 265:93-100.


Pavlov IP (1927) [Conditioned reflex]. Feldsher Akush 10:3-10.


7. Curriculum Vitae

Sarah Starosta, M.Sc. Psychology

Personal information

Date of birth 24.10.1985
Nationality German

Contact

Work address Ruhr-University Bochum
Department of Biopsychology
Faculty of Psychology
Universitätsstraße 150
44780 Bochum
Germany

Phone +49 234 322 4917
E-mail Sarah.Starosta@rub.de

Research

Grants / Scholarships

2013 Research School conference funding
2012 Grant of the “exploratory treasure” for young scientist within the DFG (German Research Foundation) Research Unit FOR1581
2010 Erasmus Scholarship
University La Sapienza, Rome, Italy

Invited talks

March 2015 Meeting of the German Neuroscience Society; Göttingen; “Breaking News Symposium”
Title: “Dynamic coding patterns in single units of the forebrain across three stages of learning”

April 2015 Laboratory of Adam Kepecs
Cold Spring Habor Laboratories, New York
Title: “Behavioral and neuronal mechanisms of extinction learning and renewal”
<table>
<thead>
<tr>
<th>Courses</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 2013</td>
<td>BCF/NWG-Course: Analysis and Models in Neurophysiology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Methods</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Electrophysiological recordings in freely moving animals; Behavioral analyses, Histology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Software Skills</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MATLAB, Spike2, MS Office, Corel Draw</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Languages</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>German (native), English (fluent), Italian (intermediate)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Teaching</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>Research practical “Introduction to programming in MATLAB”</td>
</tr>
</tbody>
</table>
| 2013     | Research practical “Influence of reward expectancy on the context-specificity of acquisition and extinction “  
|          | Research practical “Introduction to programming in MATLAB” |
| 2009/2010 | Tutor for the painting and crafts workshop on the human brain  
|          | Tutor for the seminar “learning” |

<table>
<thead>
<tr>
<th>Education</th>
<th></th>
</tr>
</thead>
</table>
| Since October 2011 | **Associate member**  
|                   | International Graduate School of Neuroscience  
|                   | Ruhr-University Bochum, Germany |
| since May 2011    | **PhD student, Prof. Dr. Dr. h.c. Onur Güntürkün**,  
|                   | Ruhr-University Bochum, Germany |
Oct 2008 – Mar 2011

**Master of Science, Psychology**  
*(grade: excellent)*,  
Ruhr-University Bochum, Germany.

M.Sc. Thesis: “*Representation of value and reinforcement in the avian brain as assessed with a generalization task*”  
Single unit recordings in the avian forebrain while freely moving animals were tested for generalization.  
Supervisors: Dr. Dr. h.c. Onur Güntürkün, Dr. rer. nat. Maik C. Stüttgen.

Jan 2010 – Mar 2010

**Research internship,**  
Instituto superiore della sanità, Rome, Italy.

Topic: “*Epigenetic influences of the early social environment on brain and behavior development*”  
Behavioral testing of stressed mice grown up in different social environments  
Supervisor: Dr. Igor Branchi

Aug 2009 – Jan 2010

**Studies abroad, Erasmus Scholarship,**  
University La Sapienza, Rome, Italy


**Bachelor of Science, Psychology**  
*(grade: very good)*,  
Ruhr-University Bochum, Germany.

B.Sc. Thesis: “*Neuronal activity of striatal neurons during classical conditioning with varying amounts of reward*”  
Single unit recordings in the striatum of head-fixed animals  
Supervisors: Dr. Dr. h.c. Onur Güntürkün, Dr. rer. nat. Jonas Rose

grade: excellent
8. List of Publications


Starosta, S., Güntürkün, O., Stüttgen, M. C., Dynamic Coding pattern in the forebrain across three stages of learning (in preparation).

Starosta, S., Lucke, S., Uenver, M., Güntürkün, O., Stüttgen, M. C., Context-specificity of acquisition and extinction learning (in preparation).
9. Acknowledgements

Eine Menge Leute haben am Gelingen dieser Arbeit beigetragen, denen ich hiermit gerne danke möchte.

Onur, danke ich für viele spannende Jahre mit viel Freiheit, Miteinbezogen werden, einfach mal machen lassen, ohne die Story aus dem Blick zu verlieren.

Sen möchte ich für konstruktive Kritik und die richtigen Fragen über die Zeit hinweg danken.

Maik: Danke für alles was ich kann und wofür du mich begeistert hast. Fürs Unterstützen und auch machen lassen. Im Speziellen für obiges Zitat, den neurophysiologischen Spaziergang inklusive Kepecs 2008 und die Begeisterung für geile Spikes! Wow!

Nils: Da der Platz begrenzt ist, gebe ichs nur zurück: Fürs Wissenschaft lieben!! Mit allem was dazu gehört !!!


Allen meinen Lieben fürs Dasein, Unterstützen und in die echte Welt zurückholen. Besondere Erwähnung gebührt hier Michi für viel Geduld und Schokolade, lange Nächte sowie fürs Waschen, Putzen und Kochen in den letzten Wochen 😊

Und last but not least danke ich meiner Mutter für die uneingeschränkte Begeisterung für alles, was ich tue. Nichts braucht man manchmal mehr.