DIPLOMARBEIT

Titel der Diplomarbeit

What does a salamander remember after winter?
The effects of hibernation on memory retention in a spatial task.

angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag. rer.nat.)

Verfasserin / Verfasser:	Anne Katrin Hloch
Matrikel-Nummer:	a0400713
Studienrichtung /Studienzweig	
(lt. Studienblatt):	Zoologie
Betreuerin / Betreuer:	Prof. Ludwig Huber

Wien, im August 2010

For my parents

DANKSAGUNG

Allen voran möchte ich meinen Eltern danken, die mir mein Studium nicht nur finanziell ermöglicht haben sondern mich auch stets in meinem Tun ermutigt und unterstützt haben.

Danke Martin, dass du immer für mich da warst. Danke, dass du jeden noch so kleinen Erfolg mit mir gefeiert hast und mir bei jedem Rückschlag zur Seite gestanden bist - mit Schokolade und auch mal einer Schulter zum Ausweinen.

Danke Jo & Stephan für eure guten Tipps, die mir durch die Höhen und Tiefen meines Studiums geholfen haben und danke für die unzähligen Daumen, die ihr in diesen 6 Jahren für mich gedrückt habt!

Bei meinen Mädls, Tami, Nadine & Christina, bedanke ich mich für jede Stunde gemeinsamen Lernens, für jedes Glas Prosecco und für jeden Lachkrampf (während unserer Vorlesungen). Danke, dass ihr mich vom ersten Unitag an auf diesem Weg begleitet habt, das werde ich euch nie vergessen! Und ein ganz spezielles Dankeschön noch einmal an dich, allerbesteste ZooCo Tami, für unsere schöne gemeinsame Biologen-WG-Zeit!

Und danke, kleine Banja, dass sich in den letzten Monaten nicht immer alles nur um die Diplomarbeit gedreht hat…!

ACKNOWLEDGEMENTS

CONTENTS

1   ABSTRACT

Hibernation causes extremely reduced synaptic activity. This is likely to have an impact on memory. Recent research in mammals suggests that they do not remember spatial or operant tasks but that social memory remains intact. This study investigated whether salamanders can remember a learned task over a period of hibernation. Fire salamanders were trained to choose the correct arm of a T-maze. After all subjects had successfully learned the task half of them were hibernated for 100 days while the others served as controls and were maintained under normal conditions during this time. A memory retention test in spring revealed that subjects from both conditions retained the learned response. Those salamanders that did not pass the strict retention criterion reached the training criterion significantly faster in the retraining than in the initial one, suggesting that at least some information was retained. Two additional tests showed that fire salamanders did not rely on olfactory cues when navigating a T-maze. Rather, they showed response learning or used a combination of different extra-maze cues, potentially including visual information or the magnetic field. These findings suggest that either the brain structure or the processes involved in hibernation differ between amphibians and mammals.

2   GENERAL INTRODUCTION

Amphibians evolved from fish and are considered the living link between their fully aquatic ancestors and the terrestrial amniotes –the mammals, birds and reptiles (Hillman, Withers, Drewes & Hillyard, 2009). After they colonized the land nearly 350 million years ago amphibians developed a wide range of different adaptations which resulted in the greatest diversity of modes of life history of any class of vertebrates (Duellman & Trueb, 1994). Research on the class of *Amphibia* is essential for our knowledge and understanding of a wide range of biological phenomena. Given their evolutionary history investigation into the cognitive abilities of amphibians can be informative about cognitive evolution and development.

There has been intense research on several aspects of amphibian biology such as development and embryology, regeneration, mating and breeding, hybridization, vocalization, acoustic communication and movement (Duellman & Trueb, 1994; Roots, 2006). However, in animal cognition amphibians are among the least studied classes of vertebrates and for a long time they were generally considered to have poor cognitive abilities (Ray, 1970; Macphail, 1982). However, more recent work has found evidence of habituation in amphibians that is very similar to that seen in other vertebrates (Macphail, 1982). Research has also found that amphibians are capable of association-formation through classical conditioning (e.g. conditioning of the nictitating membrane; Goldstein, Spies & Sepinwall, 1964). In the field of instrumental conditioning the majority of experiments have used aversive stimuli (Macphail, 1982). For example, there is evidence of instrumental avoidance learning in tiger salamanders (*Ambystoma tigrinum*). A vibratory stimulus predicted the onset of an aversive bright light in their chamber. They were able to learn to move into the other compartment on presentation of the CS vibration (Ray, 1970). Furthermore, Miller and Berk (1977) showed that both, larval and adult claw-toed frogs (*Xenopus laevis*) can be successfully trained to avoid the black end of a tank by being punished with electric shock.

Although research has focused on experiments using negative reinforcement, a few studies have shown that positive reinforcers are efficient too. Moore and Welch (1940; cited by Macphail, 1982) successfully trained larval salamanders (*Ambystoma paroticum*) to rise to the water surface in response to flashing light to receive a food reward.

Larval crested newts (*Triturus cristatus*) learned to discriminate between a black circle and a black triangle, when reinforced with a piece of worm after biting at the correct stimulus (Hershkowitz & Samuel, 1973). However, adult newts trained on the same task were unable to learn the discrimination (Hershkowitz & Samuel, 1973). Furthermore, Schmajuk, Segura and Reboreda (1980) showed that dehydrated toads (*Bufo arenarum*) can learn to make the correct choice in a Y-maze for the reward of water.

The findings of maze-studies on amphibians using negative reinforcement are ambiguous. Larval newts (*Triturus viridescens*) were successfully trained to make the correct choice in a Y-maze (Fankhauser, Vernon, Frank & Slack, 1955). Punishment for an incorrect choice was presentation of a bright light and then the larvae was sucked up into a pipette. Leopard frogs on the other hand, could not learn to escape from electric shock by jumping into another compartment of a shuttle box (McGill, 1960) which is in contrast to the findings in Woodhouse toads (*Bufo woodhousii*) that were successful in a similar task (Boice, 1970). However, McGill (1960) reported that all experimental subjects eventually died as a result of the shock which suggests that the shock caused damage to the animal and makes his results inconclusive. The general inconsistency of the amphibians´ performance in instrumental avoidance tasks does not necessarily mean that they lack learning capacity but rather that these experimental paradigms might not activate their behaviour. Electric shocks and bright light are commonly used as unconditioned aversive stimuli. However, the former can induce motor-inhibition in frogs and toads – thus preventing responses (McGill, 1960) and salamanders are known to habituate to the latter (Ray, 1970).

To sum up, there is no convincing evidence to conclude that amphibians are incapable of learning but there is a need for much more research to gain a better insight into the cognitive abilities of these animals. Therefore, this experiment presented salamanders with a T-maze task in which they were rewarded for choosing the correct arm by being given access to shelter.

Much attention has been paid to maze learning abilities of mammals, especially rats. And in contrast to the ambiguous findings in amphibians it is accepted that rats are remarkably efficient at completing maze tasks (Macphail, 1982). They are able to perform above chance even when they are presented with a radial arm maze with up to 40 arms (Cole & Chappell-Stephenson, 2003).

Although intense research has been done on amphibian movement, migration and homing behaviour (Denoel, 1996; Diego-Rasilla, Luengo & Phillips, 2008; Duellman & Trueb, 1994; Roots, 2006; Schulte, Küsters & Steinfartz, 2007; Schmidt, Schaub & Steinfartz, 2007; Sinsch, 1990, 1992) studies on small scale spatial behaviour in amphibians are scarce and the findings inconclusive. It is thought that migrating amphibians rely on olfactory, visual, acoustic and celestial cues as well as on the magnetic field or geotactic and hygrotactic information (Diego-Rasilla et al., 2008; Duellman & Trueb, 1994; Sinsch, 1992). All these different factors may be important for successful homing to the sites of reproduction, feeding and hibernation (Duellman & Trueb, 1994). However, our knowledge about homing mechanisms is fragmentary (Sinsch, 1992) and the role that these cues play in maze navigation is not clear. It is therefore important to examine what amphibians actually learn about navigating whilst in the maze and how they do so.

Within the three living groups of extant amphibians –the anurans (frogs and toads), the urodelans (salamanders) and the caecilians (worm-like amphibians that mainly live underground; Duellman & Trueb, 1994) –evidence of learning in salamanders has been particularly contradictory. While some authors have questioned the urodelans´ capacity to learn any responses (Flower, 1927; as cited in Ray, 1970; Thorpe, 1956), Fankhauser et al. (1955) found that salamanders can be comparatively efficient learners. It is therefore important to further examine the learning abilities of this group. The subject of this experiment was the local fire salamander (*Salamandra salamandra*). This nocturnal species (Nöllert, 1992) is a sit-and-wait forager (Wells, 2007). As an adult it is territorial and shows great site fidelity, sometimes remaining in the same site for many years (Rebelo & Leclair, 2003; Schmidt et al., 2007; Wells, 2007). Further, (and particularly important for this study) fire salamanders normally hibernate over winter and will sometimes use the same hibernacula for up to 20 years (Wells, 2007). In central Europe hibernation takes place from November to March (Denoel, 1996), however, this species can stay awake during warm winters (e.g. in northern Africa) without any adverse effects (Nöllert & Nöllert, 1992). In autumn fire salamanders leave their summer territory to hibernate hidden under leaves, in deep rock crevices or in caves. Annually, in spring, they return to the same summer territory (Hödl, 2009; personal communication). This suggests that their spatial memory might survive hibernation.

Hibernation is a strategy that enables animals to survive hard conditions during winter (Campbell & Reece, 2003). It is defined as "long-term torpidity to escape unfavorable winter conditions and to conserve energy, while surviving upon body fat or external food stores" (Roots, 2006). Hibernation is observed in warm-blooded animals (the mammals and the birds) as well as in the cold-blooded amphibians and reptiles (Campbell & Reece, 2003).

In mammals hibernation is a common strategy used by many rodents and bats as well as by larger animals, for example, carnivorous bears, raccoons and skunks (Roots, 2006). Several bird species (e.g. pigeons, hummingbirds and swallows) practice daily torpor –a less profound state of dormancy –but only the poorwill (*Phalaenoptilus nuttalli*) is known to exhibit true hibernation (Roots, 2006).

Although fish dormancy is thought to differ fundamentally from that of terrestrial hibernators, recent work has shown that an Antarctic fish species (*Notothenia coriiceps*) enters a hibernation-like state during winter. This state is induced by changes in the photoperiod (Campbell, Fraser, Bishop, Peck & Egginton, 2008).

In general, hibernation is characterized by glandular modification, the slowing down of metabolic rate and circulation, and a dramatic drop in body temperature and heart rate (Eckert, Randall, Burggren & French, 2002; Roots, 2006). Although the term "sleep" is commonly used when talking about an animal´s inactivity during hibernation, they should not be considered the same. Whilst there is a phase of rapid eye movement and specific brain wave patterns (REM sleep) during sleep this is not the case during torpidity (Eckert et al., 2002). In mammals there is almost no brain activity (Daan, Barnes & Strijkstra, 1991) and the entry into torpor is accompanied by a loss of 50 – 65% of synapses (Von der Ohe, Garner, Darian-Smith & Heller, 2007). As neuronal functions remain active through regular use (Kavanau, 1997) it is likely that the decrease in brain activity associated with hibernation may have negative effects on memory (Millesi, Prossinger, Dittami & Fieder, 2001).

Only a handful of experiments have investigated the effects of hibernation on memory retention in mammals. Mihailovic, Petrovic-Minic, Protic & Divac (1968) trained European ground squirrels (*Spermophilus citellus*) in a maze task. After 32 days the hibernating animals´ performance was compared to that of a non-hibernating control group.

It was found that hibernation had positive effects on the learning ability of this species. However, there was no significant difference between the memory retention in the hibernating and the non-hibernating animals. This suggests that hibernation does not have any negative effects on long-term memory. Furthermore, hibernating mantled ground squirrels (*Citellus lateralis)* which before hibernation had learned to escape a water bath by making a visual discrimination were found to have a better retention than non-hibernating controls (McNamara & Riedesel, 1973). However, these findings may not be reliable because the training situation was extremely stressful (given that five individuals died in the task). Further, the squirrels only received two torpor bouts of 11 days each.

A more recent study revealed that Belding´s ground squirrels´ (*Spermophilus beldingi*) ability to recognize previously familiar conspecifics was reduced after hibernation although they were still able to recognize their littermates (Mateo & Johnston, 2000).

Millesi et al. (2001) trained ground squirrels (*Spermophilus citellus*) in two conditioned tasks, a maze task on spatial memory and an operant task on a feeding machine in which they had to push a lever to receive a food reward. To investigate the ground squirrels´ social memory they were given a social preference test in spring. In this test the test animal was put together with an unfamiliar and a familiar but unrelated squirrel. After having successfully learned the tasks, half of the experimental subjects were hibernated for seven months while the other half stayed awake without receiving any training. In both training tasks the non-hibernating animals showed higher levels of retention compared to those animals that had hibernated. Furthermore, those squirrels that had stayed active during winter needed significantly fewer trials to relearn the tasks. However, social memory was not negatively affected by hibernation.

Millesi et al´s (2001) findings contrast with the experiment of Mihailovic et al. (1968). One possible explanation for the differences is that these two studies differ in terms of the duration of hibernation phases. While the squirrels in Millesi et al´s (2001) study hibernated for seven months –a period resembling natural conditions, Mihailovic et al. (1968) terminated hibernation after only 32 days. Moreover, Mihailovic et al. (1968) compared mean group results while Millesi et al. (2001) analyzed individual performances. Furthermore, when looking at the effects of hibernation on social memory, the findings of Millesi et al. (2001) and Mateo & Johnston (2000) are inconsistent.

It should be noted here that the Mateo & Johnston study (2000) lacks a control group. The authors tested for a difference in performance before and after hibernation within the same group of animals which makes the comparison with Millesi et al.´s findings (2001) difficult. Without a non-hibernating control group one cannot pull apart the effects of hibernation from those of a period without any training on long-term memory.

Clemens, Heldmaier & Exner (2009) found similar results to those observed by McNamara and Riedesel (1973). They showed that Alpine marmots´ (*Marmota marmota*) performance in two operant conditioning tasks was unimpaired after six months of hibernation. In order to receive a food reward, experimental subjects had to jump from one box on another or to walk through a tube. However, in this experiment there was again no non-hibernating control group. Furthermore, given that the marmots´ learning curve was not distinctive it is unclear in how far these tasks can be regarded as operant learning tasks. Both, jumping on boxes and walking through tubes are behavioural patterns that are likely to be shown regularly in the wild, e.g. when marmots jump on top of rocks to watch out for predators or when they enter the tunnels of their dens.

To conclude, very little is known about the effects of hibernation on memory retention. The findings are obviously inconsistent and therefore it remains an open question whether or not mammalian memory is retained across hibernation. However, it should be noted that Millesi et al.´s experiment (2001) is rigorously controlled whereas many of the others are not.

Millesi et al. (2001) found that in mammals there is no retention of spatial or operant memory but that social recognition is not affected by hibernation. Although many amphibians also show winter torpidity, relatively little attention has been paid to their hibernation and winter physiology (Costanzo & Lee, 2005). Further, to our knowledge nothing at all is known about the effects of hibernation on memory retention in this class of vertebrates.

It is important to point out here that there are differences between mammalian and amphibian hibernation and brain structure. As far as the mammals and birds are concerned hibernation is a controlled activity (Roots, 2006). They have the ability to regulate their body function throughout hibernation and to raise their body temperature even when it is close to freezing.

Warm-blooded animals regularly rouse from torpidity, thus hibernation in mammals and birds is not uninterrupted (Roots, 2006). However, why they do so is still a subject of much speculation. It has been suggested that the warm-blooded animals´ arousals may stimulate the immune system and allow them to defecate and urinate (Roots, 2006). Furthermore, they appear to sleep during these arousals which is thought to have positive effects on brain cell regeneration (Daan et al., 1991). Since synaptic plasticity is essential for memory formation (Duday, 2002; Martin, Grimwood & Morris, 2000) the changes within the brain during hibernation (namely degeneration and regeneration) must be considered to have an impact on memory (Clemens et al., 2009).

In mammals there are two different forms of hibernation, commonly referred to as the deep and the light sleep. Although hibernation must not be considered the same as sleep these terms will be maintained as there are no good alternatives available.

While deep sleepers –many rodents and bats –have a body temperature close to freezing and awaken slowly, the carnivores (e.g. bears) show light sleep with higher body temperature and the ability to arouse quickly. Although the forms of winter torpidity vary among mammals and birds, the major difference lies between the warm-blooded and the cold-blooded animals. (Roots, 2006)

As they are unable to produce their own warmth the cold-blooded amphibians and reptiles are totally dependent on the temperature of their surroundings (Wells, 2007). Thus, they cannot arouse from hibernation but stay torpid until temperature rises (Roots, 2006). Hibernating amphibians face two environmental stresses, a lack of food and low temperatures that make them unable to move (Hillman et al., 2009). However, the risk of dying through starvation is diminished at low temperatures because they normally have internal energy reserves and metabolism is dramatically reduced (Hillman et al., 2009). Though, as soon as the ambient temperature gets cold enough to freeze amphibians are at great risk (Hillman et al., 2009).

Amphibians use different strategies to survive subzero temperatures including freeze tolerance (e.g. in the wood frog *Rana sylvatica;* Storey, 2000) or hibernation in places where freezing is not likely to occur (e.g. in *Salamandra salamandra*; Holenweg & Reyer, 2000). For example underwater, deep in the ground underneath the frost line or under a shallow covering of soil and leaves with the snow blanket providing extra insulation (Roots, 2006).

Most land-living salamanders show terrestrial hibernation (Hillman et al., 2009). However, field data on hibernation in amphibians is rare (Holenweg & Reyer, 2000) and what actually happens below ground all winter is still unknown. While Roots (2006) reports that cold-blooded amphibians are unable to move at low temperatures and remain inactive the whole winter, there is evidence that toads and salamanders respond to decreasing soil temperature by moving deeper (Hillman et al., 2009).

When investigating the effects of hibernation on memory retention the question arises what actually happens to the brain during hibernation. In torpid mammals the neurons´ dendritic arbors retract when temperature gets low and they regrow rapidly when the animal is returning to normothermy (Von der Ohe et al., 2007). This form of temperature dependent neural plasticity results in a dramatic loss of synapses. And this is likely to cause large-scale restructuring of neural connections with each arousal from hibernation (Von der Ohe et al., 2007). Millesi et al. (2001) reported that hibernation has negative effects on memory retention in ground squirrels. Similarly, human patients recovering from hypothermia can show cognitive impairments (Walpoth et al., 1997).

The large-scale neural changes observed by Von der Ohe et al. (2007) are likely to cause loss of memory associated with hibernation and impairments after hypothermia. Although the synapses recover rapidly when returning to normothermy it is likely that not all types of information in these synapses are retained (Von der Ohe et al., 2007). But where and how memories are stored, and which types of memories are most sensitive to this structural loss during hibernation is not clear. Recent work on hibernating mammals has shown that the state of torpor is also responsible for dramatic changes of the hippocampal connectivity (Nowakowski, Swoap & Sandstrom, 2009). The hippocampus is thought to play an important role in the consolidation of spatial memory and this consolidation occurs when information is transferred from the hippocampus to cortical regions of the brain (Nowakowski et al., 2009). Thus, the authors suggest that hibernation should have an impact on spatial memory in mammals.

The amphibian brain also goes through massive changes during hibernation. However, very little research has investigated this phenomenon in amphibians. In a hibernating frog species (*Rana esculenta*) physiological events were found that are characterized by apoptotic cell death in the cerebral hemispheres of the brain (Cerri, Bottiroli, Bottone, Barni & Bernocchi, 2009).

The authors suggest that the high number of dying cells in the hibernating frogs´ cerebral hemispheres stimulates cell proliferation in other brain areas, permitting the animals to survive without brain damage. It remains an open question, however, whether these dramatic changes have an effect on amphibian memory and to our knowledge no one has ever investigated this. Furthermore, it is currently not known to what extent hibernation has the same effects on memory retention in mammals and amphibians.

There are remarkable differences in the organization of amphibian brains compared to mammals. For example mammals have a neocortex whilst amphibians do not (Campbell & Reece, 2003). The neocortex, a brain area that integrates higher cognitive functions (Campbell & Reece, 2003), is thought to play an important role in the capacity to master complex multimodal problems such as maze learning (Lashley, 1943; as cited in Macphail, 1982). It is therefore plausible that the mechanisms underlying (maze) learning in amphibians may differ from those seen in mammals. Furthermore, the medial pallium of the amphibians is considered the evolutionary antecedent of the mammalian hippocampus (Macphail, 1982; Westhoff, 2000). In mammals the hippocampus is involved in the processes of learning and memory (Westhoff, 2000). However, whether the pallium is a homology and therefore serves similar functions in the amphibian brain is currently unknown.

It should therefore be doubted that the mammalian brain model applies to amphibians (Hillman et al., 2009). Whether the different brain organization results in varying effects of hibernation on memory retention should be investigated.

To sum up, very little is known about learning capacities in salamanders. Further, the effect of hibernation on memory retention in amphibians has never been investigated. Thus, this study sought to answer several questions: Can fire salamanders learn anything? And in particular, can they be trained to navigate a T-maze with positive reinforcement? If so, will they retain the learned response over 100 days of hibernation or an equivalent period without training? Furthermore, research has not dealt with the mechanisms underlying small scale spatial behaviour in amphibians. Therefore, it is the aim of this study to shed light not only on the cognitive abilities of salamanders but also on the potential retention of their spatial memory across hibernation and on the influence of different cues on navigating a T-maze.

3   EXPERIMENT 1

3.1   INTRODUCTION

As previously mentioned, studies on maze learning in amphibians are scarce and the findings are highly ambiguous. Maze experiments were mainly using negative reinforcement, which could potentially cause problems in activating an animal´s behaviour. To our knowledge nothing is known about the maze learning abilities of any adult salamander species. This is especially interesting because some authors have questioned the salamanders´ capacity to learn (Flower, 1927; Thorpe, 1956), while others have suggested that they may be comparatively efficient learners (Fankhauser et al., 1955). Therefore, we investigated whether fire salamanders can learn to make the correct choice in a T-maze for positive reinforcement (access to a hiding place). Furthermore, if they are able to learn such a task, can they retain the learned response over 100 days of hibernation or an equivalent period without any training?

3.2   METHODS

3.2.1 Subjects

The focal species of this study were twelve captive bred one-year-old fire salamanders (*Salamandra salamandra*; Table 1). They were purchased by the University of Vienna in mid-August 2009. Due to different growth rates the salamanders differed in size, weighing between 1 and 7 g. The salamanders´ sex was not known because they had not reached sexual maturity at the time of the experiments.

The subjects were kept in two adjacent terrariums in two groups of six individuals. Although fire salamanders are highly territorial, young individuals can be housed together in groups without showing any aggression towards each other. Each terrarium measured 60 x 49.5 x 50 cm and contained a sponge matting, a water bowl and pieces of bark and plant pots for hiding. The animals were maintained at approximately 20°C (± 2°C) and provided with an UV-lamp from mid-October onwards. This allowed regulation of their light-dark-cycle (light from 6 am until 6 pm). They were given free access to food (crickets and earthworms) and the terrariums were sprayed with water three times a week to ensure a moist surrounding for the salamanders.

3.2.2 Apparatus

The apparatus was a T-maze made out of white plastic covered wood. The maze consisted of a starting area and two arms (Figure 1). In both arms of the maze a small barrier was placed in front of an opaque box (7.8×6×2.6 cm). One of the boxes had an opening that allowed the salamander to hide inside while the other one could not be entered (Figure 1). Pilot experiments revealed that the salamanders did not feed or search for food in the maze. Therefore, they were rewarded for a correct choice by getting access to a dark hiding box containing a small moist sponge. As fire salamanders are nocturnal and normally hide during the day, this type of reward was both salient and valuable to them.

The barriers in each arm (Figure 1) prevented the animals from seeing which box was open. During training the T-maze was illuminated by two lamps on each side of the apparatus and covered with a transparent Plexiglas board to prevent the animals from escaping. The whole apparatus including the reward boxes and the barriers was made of plastic to allow easy cleaning. However, during training it was never cleaned. Half of the salamanders were trained to turn right and the other half to turn left. Thus, olfactory cues of all twelve subjects were spread across the whole T-maze making navigation on the basis of individual scent marks impossible. Hence, not to clean the apparatus between trials was to discourage the salamanders from using olfactory cues.
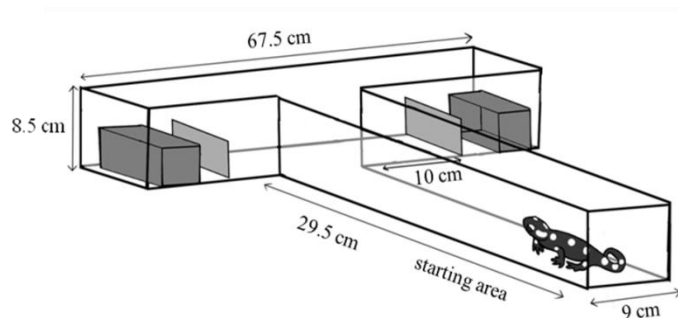

Figure 1. T-maze. Each arm contains a barrier and a box. At the beginning of each trial the experimental subject is put into the starting area.

All trials were run in a small dimly lit room without any windows. The apparatus was surrounded by white walls and a black door. The salamanders´ behaviour was observed by an experimenter who remained in the room at all times. During inter-trial intervals the subjects were kept in two holding containers (26 x 17 x 13.5 cm) in groups of six individuals. These containers had damp paper towel on the floor and a piece of bark for hiding under.

3.2.3 Procedure

The training took place between September and the end of November 2009. Prior to the onset of the experiment all salamanders were handled regularly to habituate them to human contact. In mid-September a pre-exposure phase was run during which the salamanders were put in the maze in groups of six individuals for one hour on three consecutive days to habituate them to the apparatus.

3.2.3.1 Training

Training in the spatial task started in mid-September after the pre-exposure phase had been completed. A training trial consisted of one individual being put into the starting area of the maze (Figure 1). It was allowed ten minutes to reach the goal (the reward box). Upon reaching the goal the salamander was rewarded by being allowed to stay inside the box for three minutes without any disturbance. If the salamander chose incorrectly it was allowed to remain in the maze until it either reached the goal box or ten minutes had expired. The experimenter recorded the time until first choice, the time to completion, whether the salamander completed the trial and whether its first choice was correct or not. A choice was counted as soon as the salamander touched either barrier with both forelegs. If the subject did not successfully complete the trial (did not enter the hiding box) or did not make a choice within the ten minute trial time the trial ended and the salamander was put back into the holding container. Of the twelve experimental subjects six were rewarded for choosing the right arm and the other six for choosing the left one (Table 1).

The salamanders were trained daily five days a week. The order in which they were run was randomized. Each subject received between two and four trials per day and each trial was separated by an inter-trial interval of at least 15 minutes.

The salamanders were considered to have successfully learned the task when they had completed at least 50 trials and when performance in the last 21 trials was at least 80% correct first choices. After the salamanders had successfully learned to navigate in the maze the twelve subjects were separated into two groups each containing six individuals (Table 1). One group (hibernation condition) was then hibernated under controlled conditions while the other six animals served as controls. The control animals were not put into hibernation but were kept at room temperature on a fixed light-dark-cycle for the entire hibernation period.

Three individuals of each group had been rewarded in the right, the others in the left arm (Table 1). In addition to controlling for the side of reward both groups were also counterbalanced for the speed of learning. As soon as an individual had reached criterion it was put either in the cooling chamber at 4°C the next day (hibernation condition) or back in the terrarium at approximately 20°C (control condition).

During hibernation the six salamanders were kept together in a small container (26 x 17 x 13.5 cm) containing moist paper towel and pieces of bark. Every individual of the hibernation group was hibernated for 100 days. During this time no food was available and salamanders were only handled once for cleaning. The hibernation container was sprayed with water once a week to ensure it remained moist. The control animals were maintained under exactly the same conditions as they had during training, though they were not handled except for cleaning once a month. They were kept in this manner for 100 days after they had reached criterion.

After 100 days of hibernation (hibernation condition) or 100 days under normal conditions (control condition) the fire salamanders of both groups were weighed. The animals of the hibernation group were removed from the cool room and returned to their home enclosure. They were provided with free food and were given three days to recover and become active again (during this time the control animals were maintained in a normal manner). After this, both groups were tested to see whether their spatial memory had survived (Test 1, Figure 6).

Table 1. Side of reward and type of condition for each salamander. Individuals marked with a † died during hibernation or control condition.

Salamander	Rewarded side	Condition
Merlin	left	hibernation
Stella	left	hibernation
Sashalasa †	left	hibernation
Copernicus	left	control
Cheetah †	left	control
Masimba †	left	control
Mutemwa	right	hibernation
Frank	right	hibernation
Fridolin	right	hibernation
Kamikaze	right	control
Melissa	right	control
Pinocchio	right	control

3.2.3.2 Test 1 – Memory retention test

This test examined whether the salamanders had remembered the task after hibernation (or equivalent control period). It has to be noted here that 3 salamanders died during the rest time resulting in a sample size of nine individuals, five of the hibernation group and four control animals (Table 1).

Memory retention test trials were identical to training, however, as soon as the salamander made a choice (touching a barrier with both forelegs) it was removed from the maze and did not receive differential feedback. Each salamander was placed in the maze individually and allowed ten minutes to make a choice. Both the hiding and the closed box were presented in exactly the same manner as in a training trial though the animals did not have access to them. As soon as a subject made a choice it was taken out of the maze. This prevented differential reinforcement and thus ensured the animals did not learn anything in a test trial. If no choice was made during the ten minute trial time the individual was put back in the holding container and retested later.

Each salamander received six test trials on three consecutive days; they were not run on more than two test trials per day. The subjects did not receive any training trials during this test.

3.2.4 Analysis

All analyses were run on PASW Statistics 18 (formerly called SPSS). Different groups or conditions were compared using t-tests. Data reached criterion for significance when p-values were smaller than 0.05.

3.3 RESULTS

3.3.1 Training

The performance of both, the hibernation and the control condition clearly improved over the course of the last 50 training trials. Figure 2 shows the average percentage of correct first choices presented in blocks of five trials. All individuals (n=12) reached criterion. It took the fastest animal 50 training trials and the slowest one 83 trials to reliably choose the rewarded arm. On average the salamanders reached criterion after 67.7 (SE ± 3.3) trials.
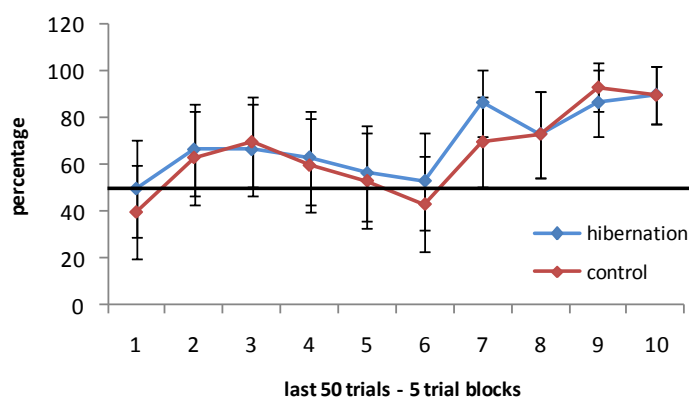

Figure 2. Percentage of correct first choices of hibernation (blue line) and control condition (red line) over the last 50 training trials. Training trials are presented in blocks of five trials. The black line represents the chance level and the whiskers the standard error.

Statistical analysis revealed that there was no significant difference in learning success between the hibernation and the control condition (t(10)= -.586, p= .571) or between the side of the rewarded arm (t(10)= -.586, p= .571).

Figure 3 shows that the average time until first choice and the average time to complete the task were constant across training trials for both conditions. It took the salamanders on average 2.38 min. (SE ± 6 sec) to make their first choice (namely touching a barrier with both forelegs) and 3.43 min. (SE ± 8 sec) to enter the hiding box and thus complete the task.
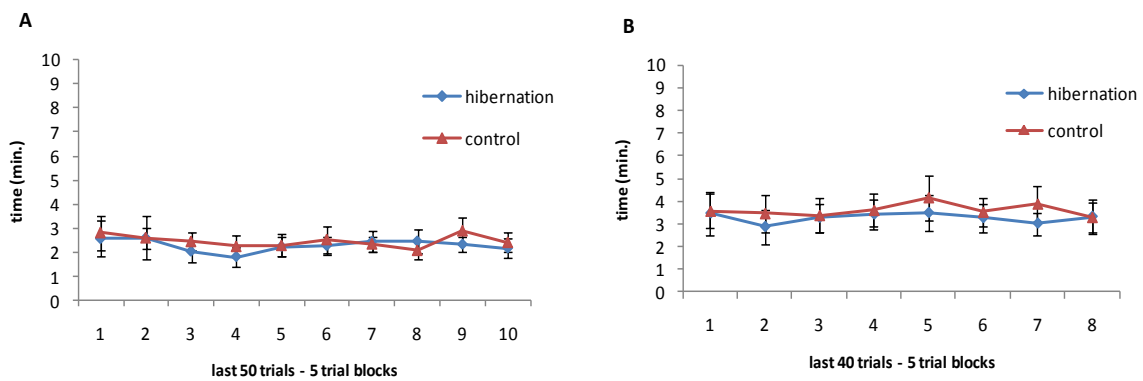

Figure 3. **A)** Average time until first choice for both, the hibernation (blue line) and the control condition (red line) over the last 50 training trials. **B)** Average time to complete the task for both conditions over the last 40 trials. Incomplete training trials are excluded here. The whiskers represent the standard error.

The performance of the animals in the two conditions did not differ in the time until first choice (t(10)= -.546, p= .597) or in the time to completion (t(10)= -1.037, p= .324). Furthermore, there was no difference between the sides of reward in the time until first choice (t(10)= -.245; p= .812) or in the time to completion (t(10)= -.640, p= .537).

3.3.2 Independent observations

The six fire salamanders of the hibernation condition were checked at least once a week and these checks revealed that– although probably torpid due to a temperature of 4°C – they were moving inside their keeping container. There is no quantitative data on these observations, however, it can be reported that the subjects clearly changed their positions within the tank from one week to the other.

Furthermore, they reacted when sprayed with water by slowly crawling elsewhere. After the hibernation period individuals were removed from the cooling chamber. After a few minutes at room temperature they were awake again and seemed to move nearly as fast as before torpidity.

3.3.3 Memory retention test

After a total of 103 days off, subjects of both conditions (five hibernating and four control animals) were run on the memory retention test. Figure 4 shows that five salamanders –three of the hibernation and two of the control condition –retained the learned response, reaching the criterion of at least 83% correct first choices (= at least five out of six test trials correct). A two-tailed binomial test with 50% chance level revealed that choosing the correct arm in six out of six trials differed significantly from chance level (p= .031). The four individuals that did so (Merlin & Stella of the hibernation group and Kamikaze & Pinocchio of the control group) are marked with a black star (Figure 4). Fridolin´s performance is not significant on the individual level (p= .219), however, Figure 4 shows that he also reached the criterion of more than 83% correct first choices. Therefore he is marked with a white, not a black star (Figure 4). Statistical analysis further showed that the hibernation and the control group do not significantly differ in the performance on the memory retention test (t(7)=-.14, p= .951). Therefore data was pooled for all further analyses.
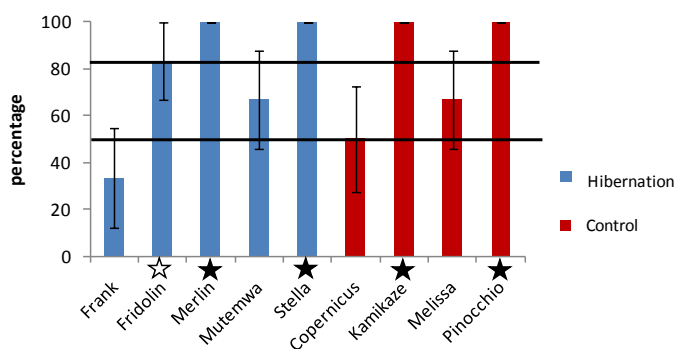

Figure 4. Results of memory retention test. The five animals that reached criterion (upper line) are marked with a star. Black stars show significance on the individual level. The lower line represents the chance level and the whiskers the standard error.

27

Overall group performance was significantly above chance level (t(8)=3.33, p= .010) and there is a trend towards significance for both, the hibernation (t(4)= 2.14, p= .099) and the control condition (t(3)= 2.33, p= .102).

3.3.4 Retraining

The four animals that did not pass the memory retention test (Frank, Mutemwa, Copernicus & Melissa; see Figure 4) were retrained until they had again reached the training criterion of more than 80% correct first choices. The number of trials to reach criterion was compared between training and retraining (Figure 5). It took these subjects on average 67.8 (SE ± 4.3) trials in the training but only 23.3 (SE ± 2.3) trials in the retraining to reach criterion. All animals (n=4) learned the maze-task significantly faster in the retraining than in the initial training (t(3)= 7.836, p= .004).
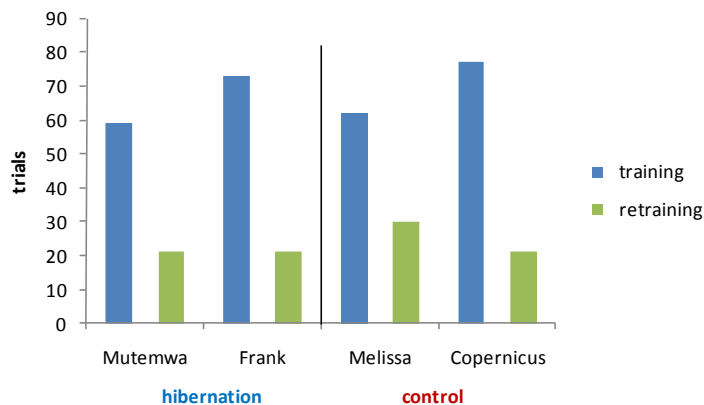
Figure 5. Number of trials until reaching criterion compared between training (blue bars) and retraining (green bars). Individuals on the left side belong to the hibernation condition, those on the right side to the control condition.

3.4   DISCUSSION

To sum up, Experiment 1 clearly showed that non-larval fire salamanders are able to learn. This is in contrast to the widespread view of the past that urodelans are cognitively poor and unable to learn (Ray, 1970). The salamanders learned to make the correct choice in a T-maze for positive reinforcement. All twelve experimental subjects reached the criterion of more than 80% correct first choices within 83 training trials.

The distinctive learning curve suggests that the dark hiding box was a salient reward and that the animals had to learn how to get to it. The side of reward had no influence on the speed of learning.

This experiment is the first evidence that an amphibian species has the capacity to retain a conditioned response over 100 days of hibernation as well as over an equivalent period without any training. Five out of nine subjects passed the memory retention test, choosing the previously rewarded arm in more than 80% of test trials. It is remarkable (given the small number of test trials) that four of the five salamanders that passed the test are significant even on the individual level. The fact that there was no significant difference between the hibernation and the control condition suggests that memory was not adversely affected by hibernation. This contrasts with the few conclusive findings in mammals.

The only reliable study found that in mammals hibernation has negative effects on the retention of spatial memory and operant conditioning (Millesi et al., 2001). However, the present study suggests that hibernation and a period without any training have similar effects on amphibian long-term memory.

Two individuals of each condition did not remember the task and had to be retrained. The training criterion was reached much faster in the retraining than in the initial one. Thus it seems plausible that at least some information was retained and that it facilitated relearning of the task.

4   EXPERIMENT 2

4.1   INTRODUCTION

Now that it is clear that fire salamanders can learn to navigate a T-maze it is of great interest to look at cues that may allow this successful navigation. In the field amphibians use a variety of different cues for orientation such as acoustic, olfactory, visual and celestial cues but also the magnetic field and other factors (Sinsch, 1992). However, it is not clear which cues amphibians actually use when inside a maze. To answer this question the experimental subjects were presented with two additional tests. The first investigated their use of olfactory cues and the second examined whether they used extra-maze cues to solve this task.

4.2   METHODS

Participants, apparatus and data analysis were the same as for Experiment 1. If the salamanders had remembered the maze-task they were given six training trials (two trials per day) to prevent a drop of performance as a result of unrewarded test trials. They were then presented with Test 2 (Figure 6). If the subjects had successfully relearned the task they were presented with Test 2 directly after they met the training criterion. Upon completing Test 2 all animals were given six training trials and then presented with Test 3 (Figure 6).
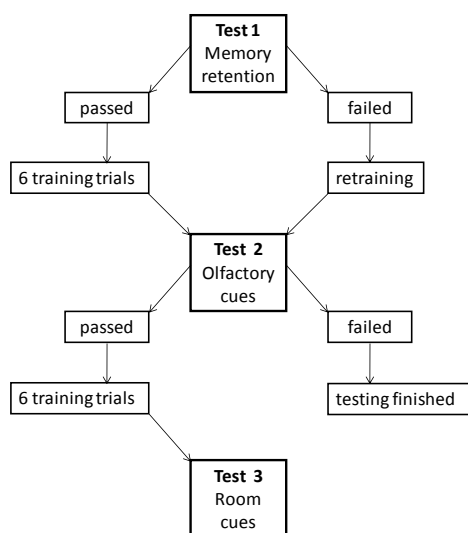

Figure 6. Overview of testing order.

4.2.1 Procedure

4.2.1.1 Test 2 – Olfactory test

This test was designed to investigate what cues the salamanders had learned about when navigating the maze. It is possible that they were able to make the correct choice by simply following the olfactory cues of the reward box or odour cues that it might have left. Therefore, both boxes were removed from the T-maze and it was cleaned between each test trial. All individuals received a total of six test trials. To prevent a drop in performance these were intermixed with normal training trials. A daily test session consisted of a training trial, followed by a test trial followed by another training trial. The test procedure was exactly the same as for the memory retention test. If the salamanders reached the criterion of five out of six correct first choices they were considered to have passed the olfactory test. If the salamanders did not pass this test they were considered to have solved the maze using olfactory cues and testing was terminated.

4.2.1.2 Test 3 – Room cue test

Test 3 examined whether the salamanders were orientating in the maze by using room cues. Thus, on test trials the maze (with both boxes in) was rotated by 180°. In all other ways test trials were identical to those given in Test 1. If the subjects used extra-maze visual cues to navigate then it was expected that they would choose the opposite side from which they had previously been rewarded in five out of six cases. However, if they chose the previously rewarded side in five out of six trials it suggests that their behavior may have been based on cues provided by their own movement or other factors.

4.3   RESULTS

4.3.1 Olfactory test

As clearly shown in Figure 7 all nine individuals chose the previously rewarded arm at least in five out of six trials resulting in a highly significant performance ($t(8)= 14$, $p< .0001$).

A two-tailed binomial test with 50% chance revealed that two hibernating animals (Fridolin & Stella) and one salamander of the control condition (Melissa) were significantly above chance on the individual level (p= .031). These subjects are therefore marked with a black star (Figure 7). White stars on the other hand are used for those individuals that did not significantly differ from chance (p= .219) but still reached the criterion of 83% correct first choices.

Both the hibernation (t(4)= 9.798, p= .001) and the control condition (t(3)= 9, p= .003) performed significantly above chance and there was no significant difference between the hibernating and the control animals (t(7)= .424, p= .685).
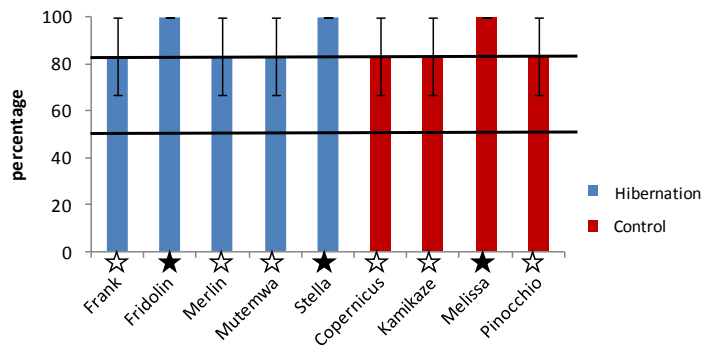
Figure 7. Results of olfactory test. All experimental subjects reached criterion (upper line) and are therefore marked with a star. Black stars show significance on the individual level. The lower line represents the chance level and the whiskers the standard error.

4.3.2 *Room cue test*

As the nine experimental subjects passed the olfactory test all of them continued with the room cue test (Figure 6). The results for this test are less clear cut. Four salamanders, one of the hibernation condition (Merlin) and three of the control condition (Copernicus, Kamikaze & Melissa) reached the criterion of at least 83% correct first choices (Figure 8). Their performance shows that they preferentially chose the actual arm that they had been rewarded with in training and that their choice was not made on the basis of extra-maze cues.

Three individuals (Merlin, Copernicus & Kamikaze) chose the correct arm in not less but 100% of test trials. A two-tailed binomial test with 50% chance showed that their performance differs significantly from chance on the individual level (p= .031). These subjects are therefore marked with a black star (Figure 8). Although Melissa reached the criterion (= at least five out of six trials correct) her performance is not significant on its own (p= .219) which is shown by using a white star (Figure 8).

In contrast to this, one hibernating individual (Fridolin) chose the previously incorrect arm in 83% of test trials suggesting that he used extra-maze cues to navigate while all the other subjects performed at about chance level.
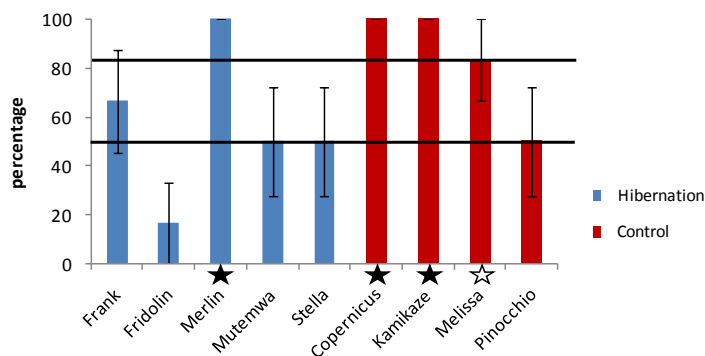
Figure 8. Results of room cue test. The four animals that reached criterion (upper line) are marked with a star. Black stars show significance on the individual level. The lower line represents the chance level and the whiskers the standard error.

Statistical analyses revealed that neither the overall group performance (t(8)= 1.890, p= .095) nor that of the hibernation condition (t(4)= .498, p= .648) or of the control condition (t(3)= 2.828, p= .066) are significantly above chance. Further, the two conditions do not significantly differ from each other (t(7)= -1.44, p= .193).

4.4   DISCUSSION

Experiment 2 clearly revealed that the fire salamanders did not use olfactory cues for navigating a T- maze. All nine animals passed the olfactory test (reaching the criterion of more than 83% correct first choices). Further, three subjects performed above chance on the individual level.

It has been reported that several salamander species neither show any territorial behaviour nor rely on scent marks of conspecifics until they reach sexual maturity (Wells, 2007). At the time of this experiment the subjects were subadult and not yet sexual mature. Hence, it is possible that they might have been too young to refer to olfactory information or that the olfactory information was not salient. Rather, fire salamanders seem to show a conditioned response (e.g. "turning left") or a response based on alternate factors either alone or maybe in combination.

Only one individual appears to have used the extra-maze visual cues for navigation while four others clearly did not use this strategy. However, given the salamanders´ behaviour, six test trials are not enough to clearly show what they actually learned about.

Migrating amphibians have been shown to use olfactory, acoustic and celestial cues as well as geotactic and hygrotactic parameters for navigation (Sinsch, 1992). However, these factors can be excluded as salient sources of information because the experimental setup controlled for them. Several amphibians have been shown to use the magnetic field for navigation (Diego-Rasilla et al., 2008). The room cue test also tested for this strategy since information gained by the magnetic field can be considered an extra-maze cue. It seems to be likely that simple response learning rather than relying on extra-maze cues has played a role for navigating the maze. However, it would have gone beyond the scope of this study to identify the underlying mechanism.

Four salamanders performed on the room cue test at about chance level. This suggests that they might have used a combination of different factors, potentially including response learning, room cues and the magnetic field. This evidence supports the idea that a number of animals adopt different strategies when navigating towards a goal depending on the nature of the problem (Pearce, 1997). If a migrating animal relies on only one single cue (e.g. a visual cue) it would get lost immediately as soon as this source of information disappears. Thus, it seems to be an adaptive strategy to use more than one cue.

5   GENERAL DISCUSSION

Experiment 1 showed that non-larval fire salamanders do have the ability to learn to navigate a T-maze when positively reinforced. Furthermore, they can retain this conditioned response over 100 days of hibernation as well as over an equivalent period without any training. Experiment 2 revealed that fire salamanders do not rely on olfactory cues when navigating a maze but rather use a combination of different factors, potentially including extra-maze cues.

Although the urodelans´ capacity to learn responses has often been questioned in the past (Ray, 1970; Macphail, 1982), our results contribute to several studies reporting successful training of salamanders with either using negative (Fankhauser et al., 1955; Miller & Berk, 1977; Ray, 1970) or positive reinforcement (Hershkowitz & Samuel, 1973; Moore & Welch, 1940; Schmajuk et al., 1980). The inconsistency in the findings on amphibian learning abilities is likely to be caused by difficulties in activating these animals´ response behaviour (McGill, 1960; Ray, 1970). Similarly, the pre-exposure phase of this study revealed that the salamanders would not feed while inside the T-maze making food an inappropriate reward. Fire salamanders are mainly sit-and-wait foragers (Wells, 2007) which could explain why they did not actively search for prey during this pre-exposure. Furthermore, given that the apparatus was comparatively brightly lit and that the study species is nocturnal and normally not active during the day (Nöllert & Nöllert, 1992), as well as the fact that shade-seeking behaviour is found in a number of different amphibian species (Wells, 2007), it seemed sensible to offer a dark hiding box as reward for choosing the correct arm. As all the experimental subjects learned to navigate the T-maze it shows that the hiding box was a salient reward for them.

After a total of 100 days of hibernation or control condition five out of nine subjects showed retention of the learned response, suggesting that fire salamanders can remember for a remarkably long time. Research on the duration of long-term memory has found that pigeons may retain learned information for as long as five years and rats for 90 days (Pearce, 1997). The short life-span of rats potentially restricts the duration of their long-term memory. However, this might not be the case in fire salamanders, a species that is known to live up to 50 years (Duellman & Trueb, 1994).

This study further showed that those subjects that failed in the memory retention test reached the training criterion (more than 80% correct first choices) much faster in the retraining than in the initial training. This finding suggests that at least some information is retained. Interestingly, there was absolutely no difference between torpid and active animals in terms of memory retention. This is in contrast to the findings in mammals.

In general, there is very little known about the effects of hibernation on memory retention and the majority of studies lack important controls. However, in Millesi et al.´s (2001) nicely controlled experiment on hibernating ground squirrels they found that the retention of spatial and operant memory is negatively affected by hibernation while there are no such effects on social recognition. Therefore we should ask why there is this difference between their findings in squirrels and ours in salamanders.

There is an important difference between mammalian and amphibian hibernation. While warm-blooded mammals show controlled torpidity, cold-blooded amphibians cannot control their body functions at low temperatures (Roots, 2006). Mammals reportedly rouse regularly during winter (Roots, 2006). Daan et al. (1991) showed that arctic ground squirrels (*Spermophilus parryii*) consistently sleep during these arousals and they suggest that sleep might have positive effects on brain cell regeneration. Nevertheless, in the alternation of torpidity and arousal the brain experiences degeneration as well as regeneration of synapses (Von der Ohe et al., 2007). It is plausible that these processes have an impact on memory retention (Clemens et al., 2009) since synaptic plasticity is essential for memory formation (Duday, 2002).

There is not much known about hibernation in amphibians (Costanzo & Lee, 2005) but it is evident that the amphibian brain also experiences dramatic neural changes during hibernation (Cerri et al., 2009; Von der Ohe et al., 2007). Whether the manner of the changes observed in mammals are comparable to those that occur in amphibians is however not clear.

There is potentially another characteristic that distinguishes torpidity in mammals from that in amphibians. Hillman et al. (2009) report that toads and salamanders are relatively active during hibernation, responding to decreasing soil temperature by moving deeper into the ground.

Although Roots (2006) states that torpid amphibians are unable to move because temperature is too low, independent observations in the course of this study contribute to Hillman et al.´s view. The fact that the fire salamanders were moving during hibernation even at 4°C could suggest that also their brains remained comparatively "active". If so, this could explain why there was no difference in memory retention between the hibernation and the control condition. However, this question cannot be answered until research investigates brain activity in hibernating amphibians.

In contrast, hibernating mammals are said to be completely inactive except during their arousals (Roots, 2006). They appear to show almost no brain activity (Daan et al., 1991), a characteristic that is likely to cause reduced memory retention (Millesi et al., 2001). Similarly, research on synaptic protein dynamics in hibernation led to the assumption that the large-scale neural changes during torpidity do not necessarily mean that all types of information are retained (Von der Ohe et al., 2007). However, which types of memories are most sensitive to structural changes of the brain during hibernation is not clear. Overall, it is plausible that the manner of hibernation has an influence on the process of memory retention and results in different findings in mammals and amphibians.

Mammals and amphibians not only differ in the type of hibernation that they experience but also in the organization of their brains. While mammals possess a neocortex, this brain area does not exist in amphibians and they lack a definitive cerebral cortex (Campbell & Reece, 2003). In mammals the neocortex is thought to play an important role in higher cognitive functions such as for example spatial reasoning and language (Macphail, 1982). Thus, it is possible that as amphibians lack a neocortex, different mechanisms underlie their spatial learning. In mammals the hippocampus is involved in the processes of learning and memory (Westhoff, 2000); in rats hippocampal "place" cells have been shown to fire when the animal passes through a specific part of a maze (O'Keefe, Burgess, Donnett, Jeffery & Maguire, 1998). Although it has been proposed that the medial pallium serves as a homologue structure (Westhoff, 2000), it is plausible that they differ mechanistically. Therefore, the differences in the organization of the brain as well as the differences in hibernation could explain the different findings in mammals and amphibians.

It is remarkable that in our experiment the hibernation and the control condition did not significantly differ in the degree of memory retention. As already mentioned above, this finding could have to do with the torpid animals being surprisingly active. Further, one cannot disentangle how the type of hibernation and the manner of the underlying learning mechanism influence long-term memory. But, no matter what the reason, the retention of memory in salamanders of both conditions serves a clear function. Fire salamanders as well as many other amphibians are highly territorial and sometimes use the same site for many years (Rebelo & Leclair, 2003; Schmidt et al., 2007; Wells, 2007). In general, amphibians often show homing to the sites of reproduction, feeding and hibernation (Duellman & Trueb, 1994), therefore it is reasonable to retain spatial information over a period of time. Fire salamanders can either hibernate or stay awake without coming to any harm, depending on the severity of the winter (Nöllert & Nöllert, 1992). Therefore it might be of great ecological relevance to remember spatial information independent of how winter is spent. However, for mammals, especially when living in a group, the retention of social recognition could be much more important than that of spatial information since their hibernacula are normally part of their home ranges (Roots, 2006).

This study demonstrates without a doubt that non-larval fire salamanders can learn responses. In particular, there is now evidence that they have the ability to navigate a T-maze for positive reinforcement. However, which mechanisms they use to do so is not clear. Experiment 2 tested for at least two possibilities, for the use of olfactory and room cues. The olfactory test clearly showed that none of the subjects used scent trails or olfactory cues from the reward box to successfully navigate the maze. This could have two different reasons. First of all, at the outset the maze was never cleaned to disperse salamander odour all over the apparatus, thus discouraging the subjects from using olfactory cues during the initial training. Secondly, it is known from other salamander species (*S. lanzai* & *S. atra*) that their territorial behaviour and scent-marking develops just before they reach sexual maturity (Wells, 2007). Further, as subadults they show only little reaction to odours of other salamanders (Wells, 2007). Given that fire salamanders reach sexual maturity at the age of two to four years (Nöllert & Nöllert, 1992) it is very likely that the one-year-old subjects were too young to rely on olfactory cues of conspecifics and rather used other sources of information.

The results of the room cue test, when the maze was rotated by 180°, were inconsistent. Four out of nine individuals were significant in choosing the previously rewarded arm showing that at least these four salamanders did not use room cues for navigation. In contrast, one subject preferred the previously unrewarded arm, suggesting that it relied on extra-maze cues to navigate to the goal. The other four salamanders performed at about chance levels which suggests that they might have used a combination of extra-maze cues and other factors to reach the goal. When animals navigate towards a goal, they often refer to different sources of information and adopt different strategies depending on the nature of the problem that confronts them (Pearce, 1997). A migrating animal that relies on one single cue (e.g. a visual cue) is at great risk to get lost as soon as this source of information disappears. Hence, using a combination of different cues to choose the correct arm of a T-maze is likely to be more efficient.

According to Sinsch (1992) a wide range of factors are involved when amphibians are migrating, including olfactory, visual, acoustic and celestial cues but also the magnetic field as well as geotactic and hygrotactic information. The experiments were run on a flat table in a room without any windows and therefore we can exclude acoustic, celestial and geotactic cues. The hiding box contained a moist sponge, however, during the olfactory test both, the boxes and the sponge were removed. Although there was no longer hygrotactic or olfactory information available the subjects continued to choose the correct arm suggesting that this types of information did not play any role in controlling their maze behaviour.

There is evidence that the palmate newt (*Lissotriton helveticus*), a salamander species, is able to orient in the homeward direction by night using no more but a magnetic compass (Diego-Rasilla et al., 2008). According to these findings one can suggest also the fire salamander to have the ability to use the magnetic field for navigation. However, information gained by the magnetic field could be considered an extra-maze cue which the room cue test controlled for.

Only one individual seemed to rely on extra-maze cues while the others used other strategies. This could be due to the experimental setup. It seems that neither the T-maze nor its surrounding, white walls and a black door, offered salient landmarks. But what was controlling the behaviour of the animals that did not use room cues but still performed above chance?

It is likely that the salamanders actually learned to turn either left or right by associating the reward with the motor response rather than by relying on any external cues. Whether this association was formed via reafference signals, motor feedback or any other mechanism is however not clear. Reafferences are stimuli produced by the own body´s muscular activity (Von Holst & Mittelstaedt, 1950). It has been suggested that motor signals from the central nervous system to the periphery (efferences) are copied and that this so-called efference copy is then used to distinguish reafferences from externally produced sensory stimuli (exafferences; Von Holst & Mittelstaedt, 1950). Besides the reafference-principle it is also known that a moving animal´s peripheral proprioreceptors (e.g. receptors of muscles and joints) receive information about a number of parameters of this movement, e.g. a limb´s position, its speed and its acceleration (Dudel, Menzel & Schmidt, 2001). This information is then projected to the central nervous system, a process called motor feedback (Dudel et al., 2001). However, it cannot be answered which mechanism was actually involved when the salamanders learned the conditioned response.

Unfortunately six test trials in the room cue test are not enough to clearly determine the strategy used for successful maze navigation. However, although the salamanders obviously relied on different ones, it is remarkable that they retained the learned response. No pattern of cue use could account for the salamanders´ ability to remember the discrimination after 100 days of hibernation or rest.

The aim of this study was to shed light on the learning abilities of amphibians and on the effects of hibernation on memory retention. Although these are attractive areas of biological research, almost nothing is known about either of these fields. Furthermore, it has to be noted that the few studies dealing with cognitive abilities in amphibians are highly contradictory. The same is true for reports on the effects of hibernation on memory. The reliability of many of these studies should be questioned given that they lack important controls.

Experiment 1 clearly revealed that fire salamanders can learn to navigate a T-maze. This finding is novel in itself. Further, we are the first to show that amphibians have the ability to retain a conditioned response over 100 days of hibernation or an equivalent period without any training. The second experiment partially disentangled which cues are used for navigating the maze. However, as with many pioneering studies, many questions arose from the research findings.

First, what are the units of the fire salamanders´ spatial abilities? Can they learn to navigate a more complex spatial task, e.g. two turns in succession or a radial arm maze? Secondly, it has to be clarified to what extent territoriality influences maze learning and the retention of spatial memory. At the time of the experiments the subjects had not reached sexual maturity and did not show any territorial behaviour. In adult fire salamanders territoriality is more pronounced (Wells, 2007), hence, it would be interesting to test adult salamanders in an identical task.

Further, it would be informative to compare the performance of fire salamanders on maze learning with that of another amphibian species. Similar findings could suggest that this ability is widespread among the whole class of amphibians. As far as amphibians are concerned, it is not clear which mechanisms underlie maze learning and which strategies are used for navigation. Recent results with reptiles (Wilkinson, Chan & Hall, 2007; Wilkinson, Coward & Hall, 2009; Mueller, Wilkinson, Hall & Huber, submitted) have found that the mechanisms controlling their spatial search behaviour are different from those observed in mammals and birds. Therefore, further experiments should elucidate the processes controlling the spatial search behaviour of amphibians.

Since little is known about the general cognitive abilities of amphibians further research with a variety of different learning experiments is essential. It would be of great interest to examine whether salamanders are also efficient in learning a completely different task, e.g. a visual discrimination. If so, would the degree of long-term memory retention be the same as was seen in a spatial task?

In general, the reports about hibernation and its effects on memory retention are scarce and inconsistent. Thus, it is evident that further research is needed to shed light on all the aspects of amphibian torpidity. For example, which kind of information do hibernating animals actually retain and which is lost? Similarly, the limits of memory retention have not yet been investigated, both in terms of hibernation and also in terms of control animals. Research on the physiological adaptations of hibernating animals has numerous potential applications for human medicine, e.g. for the treatment of hypothermia (Roots, 2006). Further, the knowledge about anti-freeze compounds found in freeze-tolerant amphibians could potentially extend the life of human organs for transplant surgery (Storey & Storey, 2004). However, this is beyond the scope of these experiments.

The current study revealed without a doubt that fire salamanders have the ability to learn to navigate a T-maze when positively reinforced, a finding that is novel in itself. Further, it has been shown here for the first time that both, hibernating salamanders and non-hibernating control animals can retain this learned response over a period of 100 days, suggesting that the duration of their long-term memory is remarkable. Moreover, it was found that the experimental subjects did not rely on olfactory cues when navigating the maze. Rather, it is likely that fire salamanders show response learning or that they use a combination of extra-maze cues, two strategies which both seem to be efficient to reach the goal.

REFERENCES

Boice, R. (1970). Avoidance learning in active and passive frogs and toads. *Journal of Comparative and Physiological Psychology, 70*, 154-156.

Campbell, H. A., Fraser, K. P. P., Bishop, C. M., Peck, L. S., & Egginton, S. (2008). Hibernation in an Antarctic Fish: On Ice for Winter. *PLoS ONE, 3,* 3.

Campbell, N. A. & Reece, J. B. (2003). *Biologie*. Berlin: Spektrum Akademischer Verlag GmbH Heidelberg.

Cerri, S., Bottiroli, G., Bottone, M. G., Barni, S., & Bernocchi, G. (2009). Cell proliferation and death in the brain of active and hibernating frogs. *Journal of Anatomy*, *215*, 124-131.

Clemens, L. E., Heldmaier, G., & Exner, C. (2009). Keep cool: Memory is retained during hibernation in Alpine marmots. *Physiology & Behavior*, *98*, 78-84.

Cole, M. R. & Chappell-Stephenson, R. (2003). Exploring the limits of spatial memory in rats, using very large mazes. *Learning and Behavior*, *31*, 349-368.

Costanzo, J. P., & Lee, R. E. (2005). Cryoprotection by urea in a terrestrially hibernating frog. *The Journal of Experimental Biology*, *208*, 4079-4089.

Daan, S., Barnes, B., & Strijkstra, A. M. (1991). Warming up for sleep?—Ground squirrels sleep during arousals from hibernation. *Neuroscience Letters*, *128*, 265-268.

Denoel, M. (1996). Phenology and home range of the fire salamander *Salamandra salamandra* terrestris (Amphibia, Caudata) in a wood of the Pays de Herve (Belgium). *Cahiers d´ethologie fondamentale et apliquee, animale et humaine*, *16*, 291-306.

Diego-Rasilla, F. J., Luengo, R. M., & Phillips, J. B. (2008). Use of a Magnetic Compass for Nocturnal Homing Orientation in the Palmate Newt, *Lissotriton helveticus. Ethology, 114*, 808-815.

Duday, Y. (2002). Molecular bases of long-term memories: a question of persistence. *Current Opinion in Neurobiology*, *12*, 211-216.

Dudel, J., Menzel, R., & Schmidt, R. F. (2001). *Neurowissenschaft: Vom Molekül zur Kognition*. Heidelberg: Springer-Verlag Berlin.

Duellman, W. E., & Trueb, L. (1994). *Biology of Amphibians*. Baltimore: Johns Hopkins University Press.

Eckert, R., Randall, D., Burggren, W., & French, K. (2002). *Tierphysiologie*. Stuttgart: Georg Thieme Verlag.

Fankhauser, G., Vernon, J. A., Frank, W. H., Slack, W. V. (1955). Effect of Size and Number of Brain Cells on Learning in Larvae of the Salamander, *Triturus viridescens. Science, 122*, 692-693.

Goldstein, A. C., Spies, G., & Sepinwall, J. (1964). Conditioning of the nictitating membrane in the frog, *Rana p. pipiens. Journal of Comparative and Physiological Psychology*, *77*, 212-220.

Hershkowitz, M., & Samuel, D. (1973). The retention of learning during metamorphosis of the crested newt (Triturus cristatus). *Animal Behaviour*, *21*, 83-85.

Hillman, S. S., Withers, P. C., Drewes, R. C., & Hillyard, S. D. (2009). *Ecological and Environmental Physiology of Amphibians*. New York: Oxford University Press.

Holenweg, A. K., Reyer, H. U. (2000). Hibernation behavior of Rana lessonae and R. esculenta in their natural habitat. *Oecologia*, *123*, 41–47.

Kavanau JL (1997) Memory, sleep and the evolution of mechanisms of synaptic efficacy maintenance. *Neuroscience*, *79*, 7-44.

Macphail, E. M. (1982). *Brain and intelligence in vertebrates*. Oxford: Oxford
University Press.

Martin, S. J., Grimwood, P. D., & Morris, R. G. (2000). Synaptic plasticity and
memory: an
evaluation of the hypothesis. *Annual Review of Neuroscience*, *23*, 649–711.

McGill, T. (1960). Response of the leopard frog to electric shock in an escape learning
situation. *Journal of Comparative and Physiological Psychology*, *53*, 443-445.

McNamara, M. C., & Riedesel, M. L. (1973). Memory and Hibernation in *Citellus
lateralis. Science*, *179*, 92-94.

Mihailovic, L., Petrovic-Minic, B., Protic, S., & Divac, I. (1968). Effects of Hibernation
on Learning and Retention. *Nature*, *218*, 191-192.

Miller, R. R., & Berk, A. M. (1977). Retention over Metamorphosis in the African
Claw-Toed Frog. *Journal of Experimental Psychology-Animal Behavior
Processes*, *3*, 343-356.

Millesi, E., Prossinger, H., Dittami, J. P., & Fieder, M. (2001). Hibernation Effects on
Memory in European Ground Squirrels (Spermophilus citellus). *Journal of
Biological Rhythms*, *16*, 264.

Mueller, J. S., Wilkinson, A., Hall, G. & Huber L. (submitted). Response-based
navigation in the jeweled lizard (*Lacerta lepida*). *Journal of Comparative
Psychology.*

Nowakowski, S. G., Swoap, S. J., & Sandstrom, N. J. (2009). A single bout of torpor in
mice protects memory processes. *Physiology & Behavior*, *97*, 115-120.

Nöllert, A., & Nöllert, C. (1992). *Die Amphibien Europas: Bestimmung, Gefährdung,
Schutz.* Stuttgart: Franckh-Kosmos Verlag.

O'Keefe, J., Burgess, N., Donnett, J. G., Jeffery, K. J., & Maguire, E. A. (1998). Place cells, navigational accuracy, and the human hippocampus. *Philosophical Transactions of the Royal Society*, *353*, 1333-1340.

Pearce, J. M. (1997). *Animal Learning and Cognition*. East Sussex: Psychology Press.

Ray, A. J. (1970). Instrumental avoidance learning by the tiger salamander *Ambystoma tigrinum. Animal Behaviour*, *18*, 73-77.

Rebelo, R. & Leclair, M. H. (2003). Site tenacity in the Terrestrial Salamandrid *Salamandra salamandra. Journal of Herpetology*, *37*, 440-445.

Roots, C. (2006). *Hibernation*. Westport: Greenwood Press.

Schmajuk, N. A., Segura, E. T., & Reboreda, J. C. (1980). Appetitive conditioning and discriminatory learning in toads. *Behavioral and Neural Biology*, *28*, 392-397.

Schmidt, B. R., Schaub, M., & Steinfartz, S. (2007). Apparent survival of the salamander Salamandra salamandra is low because of high migratory activity. *Frontiers in Zoology*, *4*, 19.

Schulte, U., Küsters, D., Steinfartz, S. (2007). A PIT tag based analysis of annual movement patterns of adult fire salamanders (*Salamandra salamandra*) in a Middle European habitat. *Amphibia-Repilia*, *28*, 531-536.

Sinsch, U. (1990). Migration and orientation in anuran amphibians. *Ethology, Ecology & Evolution*, *2*, 65-79.

Sinsch, U. (1992). *Animal Homing*. London: Chapman & Hall Animal Behaviour Series.

Storey, K. B. (2000). Vertebrate Freeze Tolerance: Molecular Studies of Signal Transduction and Gene Expression. In G. Heldmaier & M. Klingenspor (Eds.), *Life in the cold. Eleventh International Hibernation Symposium*. Heidelberg: Springer-Verlag Berlin.

Storey, K. B. & Storey, J. M. (2004). Physiology, Biochemistry, and Molecular Biology of Vertebrate Freeze Tolerance: The Wood Frog. In B. J. Fuller, N. Lane & E. E. Benson (Eds.), *Life in the Frozen State*. Florida: CRC Press LLC.

Thorpe, W. H. (1956). *Learning and Instinct in Animals*. Cambridge: Harvard University. Press.

Von der Ohe, C. G., Garner, C. C., Darian-Smith, C., & Heller, H. C. (2007). Synaptic Protein Dynamics in Hibernation. *The Journal of Neuroscience*, *27*, 84-92.

Von Holst, E., & Mittelstaedt, E. (1950). Das Reafferenz-Prinzip. *Naturwissenschaften, 37,* 464–476.

Walpoth, B. H., Walpoth-Aslan, B. N., Mattle, H.P., Radanov, B. P., Schroth, G., Schaeffler, L., Fischer, A. P., Von Segesser, L., & Althaus, U. (1997). Outcome of survivors of accidental deep hypothermia and circulatory arrest treated with extracorporeal blood warming. *The New England Journal of Medecin*, *337*, 1500-1505.

Wells, K. D. (2007). *The Ecology and Behavior of Amphibians*. London: The University of Chicago Press.

Westhoff, G. (2000). *Berichte aus der Biologie. Das mediale Pallium der Amphibien: Eine vergleichende anatomische Studie an Discoglossus pictus (Ordnung: Anura) und Plethodon jordani (Ordnung: Caudata).* Aachen: Shaker Verlag.

Wilkinson, A., Chan, H. & Hall, G. (2007). Spatial Learning and Memory in the Tortoise (*Geochelone carbonaria*). *Journal of Comparative Psychology*, *121*, 412–418.

Wilkinson, A., Coward, S. & Hall, G. (2009). Visual and response-based navigation in the tortoise (*Geochelone carbonaria*). *Animal Cognition*, *12*, 779–787.

APPENDIX

SUMMARY

Hibernation is an adaptive strategy that allows animals to survive the harsh environmental conditions that are experienced during winter (Campbell & Reece, 2003). Warm-blooded animals (the mammals and the birds) and the cold-blooded reptiles and amphibians are known to hibernate (Roots, 2006). Hibernation is characterized by low body temperature and a dramatic reduction of metabolic and neuronal functions (Eckert et al., 2002). There is almost no brain activity during hibernation (Daan et al., 1991). This is likely to have large effects on memory retention as neuronal connections stay functional through regular use (Kavanau, 1997).

So far these effects have only been investigated mainly in mammals (e.g. squirrels, Mihailovic et al., 1968; Mateo et al., 2000; Millesi et al., 2001; marmots, Clemens et al., 2009). However, the results are inconsistent and the majority of studies lack proper controls. The only rigorously controlled study suggests that there is no retention of spatial memory and operant conditioning in hibernating squirrels but that social recognition is not negatively affected by hibernation (Millesi et al., 2001). To our knowledge no one has ever investigated memory retention across hibernation in any non-mammalian species.

Amphibians are an interesting case as many species survive the cold winter by hibernating (Nöllert & Nöllert, 1992). Furthermore, in animal cognition amphibians are among the least studied classes of vertebrates (Ray, 1970). Traditionally they have been considered to be poor learners (Ray, 1970; Thorpe, 1956). However, there is now good reason to assume that they can learn operant responses (Boice, 1970; Fankhauser et al., 1955; Hershkowitz & Samuel, 1973; Miller & Berk, 1977; Moore & Welch, 1940; Ray, 1970; Schmajuk et al., 1980). Though, again the findings are inconsistent. Negative results can potentially be caused by problems with the experimental setups which may not activate the amphibians` behaviour.

The aim of this study was to examine the fire salamander´s (*Salamandra salamandra*) ability to learn a maze-task. Furthermore, having learned the maze the effects of hibernation on memory retention were examined. Since these salamanders are known to return to the same summer territory after hibernation (Wells, 2007), it is likely that their spatial memory – in contrast to that of mammals- might survive hibernation. Therefore, twelve one-year-old animals were trained to choose the correct arm in a T-maze.

After they successfully learned the task half of them were hibernated for 100 days while the others served as controls and remained under normal keeping conditions. In spring all experimental subjects were tested for the retention of memory. Subjects that did not retain the conditioned response were retrained. A second experiment investigated what cues they had actually learned about in the maze. All salamanders were run in two additional tests, one that controlled for olfactory cues and one that examined the use of room cues.

All subjects reached the training criterion of more than 80% correct first choices, revealing that salamanders can learn to navigate a T-maze. Furthermore, more than half of the animals passed the memory retention test showing that fire salamanders can retain a learned response over 100 days of hibernation or an equivalent period without any training. Those salamanders that had forgotten the task relearned it significantly faster in the retraining than in the initial training indicating that at least some information was retained. Interestingly, there was no difference in the degree of retention between the hibernation and the control condition. The results of the two additional tests suggest that this species does not rely on olfactory information when navigating a T-maze. Rather, they seem to show response learning or they use a combination of different extra-maze factors, these could potentially include visual cues and the magnetic field.

These findings contribute to the view that amphibians are efficient learners. The fact that the information was retained in both conditions contrasts with the few findings in mammals. Mammals and amphibians differ in the manner of hibernation (Roots, 2006) and the organization of the brain (Campbell & Reece, 2003). It is therefore suggested that the inconsistent findings might be the result of differences between these two classes of vertebrates. In general, there is very little known about hibernation and its effects on long-term memory in the wide range of animals that show torpidity.

To conclude, this study showed that it is important to investigate aspects of amphibian cognition and biology and the interactions between the two. It is particularly important to further acknowledge amphibian cognition and behaviour because they are considered the living link between the aquatic fish and the terrestrial amniotes (Hillman et al., 2009). This knowledge can contribute to our understanding of cognition in all classes of vertebrates.

ZUSAMMENFASSUNG

Winterschlaf (Hibernation) ist eine adaptive Strategie, die es Tieren erlaubt, die harten Umweltbedingungen während des Winters zu überleben (Campbell & Reece, 2003). Sowohl Warmblüter (Säugetiere und Vögel) als auch die kaltblütigen Reptilien und Amphibien halten Winterschlaf (Roots, 2006). Er ist charakterisiert durch geringe Körpertemperatur und dramatische Reduktion des Stoffwechsels sowie der neuronalen Funktionen (Eckert et al., 2002). Während der Hibernation zeigt das Säugerhirn so gut wie keine Aktivität mehr (Daan et al., 1991). Da neuronale Verbindungen durch ihren regelmäßigen Gebrauch funktionstüchtig bleiben, könnte dieser Zustand das Erinnerungsvermögen beeinflussen (Kavanau, 1997).

Bisher wurden die möglichen Effekte von Winterschlaf auf das Gedächtnis hauptsächlich an Säugetieren untersucht (Mihailovic et al., 1968; Mateo et al., 2000; Millesi et al., 2001; Clemens et al., 2009). Allerdings sind die Ergebnisse widersprüchlich und dem Großteil der Studien fehlt es an angemessenen Kontrollen. Die einzige sorgfältig kontrollierte Studie lässt aber vermuten, dass sich Säuger nach dem Winterschlaf weder an räumliche Informationen noch an operante Konditionierung erinnern, während das Wiedererkennen bekannter Artgenossen vom Winterschlaf nicht negativ beeinflusst wird (Millesi et al., 2001).

Auch Amphibien halten verbreitet Winterschlaf (Nöllert & Nöllert, 1992). Darüber hinaus zählen sie zu den am wenigsten erforschten Wirbeltieren innerhalb der Kognitionsbiologie (Ray, 1970) und wurden ursprünglich als nicht lernfähig angesehen (Ray, 1970; Thorpe, 1956). Heutzutage gibt es jedoch guten Grund anzunehmen, dass Amphibien durchaus Reaktionen erlernen können (Boice, 1970; Fankhauser et al., 1955; Hershkowitz & Samuel, 1973; Miller & Berk, 1977; Moore & Welch, 1940; Ray, 1970; Schmajuk et al., 1980). Allerdings sind auch hier die Ergebnisse nicht immer eindeutig, möglicherweise ein Resultat oft unausgereifter Versuchstechniken, die das Verhalten der Tiere nicht aktivieren konnten.

Ziel der vorliegenden Studie war es, Rückschlüsse über die Lernfähigkeit von Feuersalamandern (*Salamandra salamandra*) in Labyrinthen zu erhalten und ferner, die Effekte von Winterschlaf auf das Gedächtnis dieser Amphibienart zu untersuchen. Feuersalamander kehren jährlich nach dem Winterschlaf in dasselbe Sommerterritorium zurück (Wells, 2007). Es ist daher naheliegend, dass ihr räumliches Gedächtnis –im Gegensatz zu dem der Säuger –den Winterschlaf überdauert. Zwölf einjährige Tiere wurden trainiert, den korrekten Arm eines T-förmigen Labyrinthes (T-maze) auszuwählen.

Nach dem Erlernen dieser Aufgabe wurde die eine Hälfte der Salamander für 100 Tage überwintert während die anderen wach blieben und als Kontrollgruppe dienten. Im Frühling wurden alle Tiere auf ihr Erinnerungsvermögen getestet (memory retention test). Jene Salamander, die die konditionierte Reaktion nicht mehr zeigten, wurden erneut trainiert. Um zu untersuchen, was die Amphibien tatsächlich über die Aufgabe gelernt hatten, wurden sie zusätzlich auf Orientierung anhand olfaktorischer (olfactory test) und räumlicher Informationen (room cue test) getestet.

Alle Tiere erreichten das Trainingskriterium von mehr als 80% korrekten Erstentscheidungen. Dies zeigt deutlich, dass Feuersalamander lernen können, sich in einem T-maze zu orientieren. Weiters bestanden mehr als die Hälfte der Salamander den „memory retention test", ein Beweis dafür, dass sich diese Art sowohl nach 100 Tagen Winterschlaf als auch nach einer äquivalenten Zeitspanne ohne Training noch an die erlernte Reaktion erinnern kann. Jene Tiere, die die erlernte Aufgabe vergessen hatten, erlernten sie signifikant schneller als im ursprünglichen Training. Dies könnte darauf hindeuten, dass zumindest ein Teil der Information gespeichert bleibt. Die Ergebnisse des „olfactory test" legen nahe, dass Feuersalamander bei der Navigation in einem T-förmigen Labyrinth nicht auf olfaktorische Signale angewiesen sind. Vielmehr dürften sie eine konditionierte Reaktion zeigen oder aber die Aufgabe mit Hilfe einer Kombination mehrerer Faktoren, wie etwa räumlicher Information oder dem Magnetfeld, lösen.

Die hier gewonnenen Erkenntnisse sind ein neuerlicher Beweis für die Effizienz, die Amphibien beim Erlernen von Reaktionen an den Tag legen können. Die Tatsache, dass sich sowohl die Winterschlaf- als auch die Kontrollgruppe im Frühling noch an die erlernte Information erinnerten, steht im Widerspruch zu den Erkenntnissen aus Säugetierstudien. Säugetiere und Amphibien unterscheiden sich in der Art und Weise des Winterschlafes (Roots, 2006) sowie in der Organisation ihres Gehirns (Campbell & Reece, 2003). Es liegt daher die Vermutung nahe, dass die widersprüchlichen Ergebnisse aus diesen Unterschieden resultieren könnten. Da generell über Winterschlaf nur sehr wenig bekannt ist, wäre zusätzliche Forschungsarbeit notwendig, um die Effekte des Phänomens Hibernation auf das Langzeitgedächtnis zu klären.

Amphibien gelten als die lebende Verbindung zwischen den aquatischen Fischen und den landlebenden Amniontieren (Hillman et al., 2009). Zusammenfassend lässt sich daher sagen, dass die Erforschung sowohl der kognitiven Fähigkeiten als auch der allgemeinen Biologie der Amphibien zu unserem Wissen über alle Wirbeltierklassen beitragen kann.

DECLARATION

I assure that I wrote this Diploma thesis independently, using only the indicated sources and aids. Places and passages inferred from other factories literally or according to the sense are marked under specification of sources as are pictures, tables and figures.

ERKLÄRUNG

Ich erkläre, dass ich diese Diplomarbeit selbstständig verfasst, und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Alle Stellen dieser Arbeit, die im Sinne oder Wortlaut anderen Werken entnommen wurden, sind unter Angabe der Quelle kenntlich gemacht. Selbiges gilt für alle Fotos und Darstellungen.

Vienna, August 30th 2010

Anne Katrin Hloch

CURRICULUM VITAE

Personal Data

Name   Anne Katrin Hloch

Nationality   Austria

Date of Birth   11th February 1986

Education

1992-1996   Volksschule Aschach an der Steyr

1996-2004   Bundesgymnasium Steyr Werndlpark

2004   School leaving certificate: Matura

                Grading: 1.0

Studies

2004-2006   Study of Biology, Introductory Phase

Since 2006   Study of Biology, Deepening Phase
                Department of Zoology, University of Vienna, Vienna, AUT.

Since 2009   Diploma Thesis
                Department of Cognitive Biology, University of Vienna, Vienna,
                AUT.

                Thesis entitled "What does a salamander remember after winter?
                The effects of hibernation on memory retention in a spatial task."

                Referee: Ao. Univ.-Prof. Mag. Dr. Ludwig Huber