Towards Automated Training of Mice for a Bimodal Attention Task

by

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Submitted in partial fulfillment of the requirements of the degree
of Master of Science in Interdisciplinary Neuroscience

in the Faculty of Biosciences

Goethe University Frankfurt am Main

June 2018

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3. Summary

Training time for difficult cognitive tasks is at present the major bottleneck for investigation of higher brain functions in mice. In addition, experimenter-controlled, manual training suffers from other downsides such as restricting water or food intake, imposing the time of day and duration of the training onto the animals, and handling stress. An automated training system that provides the animals with access to training around the clock while monitoring the behavior and allowing for individually tailored training schedules can mitigate the aforementioned problems.

Here, I developed a social home cage system for housing up to 6 mice which provides RFID-controlled access to a two alternative forced choice task (2AFC) using audiovisual cues. Voluntary access to the training station led to animals performing many more trials per day compared to manual training, and receiving close to their ad libitum water intake. The animals were significantly more active during the dark cycle and had a minimum activity during mid light cycle (afternoon). I examined several training strategies to train the mice for the 2AFC task with visual and different auditory stimuli. After training the animals in this apparatus, I started daily manual sessions with intermittent inactivation of auditory cortex using optogenetics in order to explore the role of auditory cortex in discrimination of auditory stimuli and to provide proof of concept for the transferability of the automated training to the manual experiments addressing biological questions. In addition, I examined training the mice for a bimodal attention task with alternating blocks of auditory and visual stimulus. This system thus provides a promising avenue for acquisition of complex tasks, while enhancing the welfare of the animals by keeping them in a social and more spacious cage, and without long-term water restriction.

4. Introduction

The experimental toolbox for investigating neural circuits in the mouse brain has developed vastly within the last decade. Genetic tools and viral vectors for mice give us unprecedented capabilities to explore the neural circuits that are not available for any other species as close to humans in terms of evolution. Among those are tools to excite, inhibit, label, image and record the activity of defined populations of neurons depending on their gene expression or location in the brain.

However in order to understand the function of a neural circuit we need to test the circuit while it is performing a function, and arguably the only quantifiable brain function in animals is behavior. For example patch clamping (either in vivo or vitro), or electrode recordings of a mouse who is passively hearing a sound stimulus, can give us a lot of information about the properties of the neurons being recorded, but the full picture can not be drawn without exploring those neurons while they are processing the stimuli that are relevant to the current state of the animal. A complex neural system which is continuously bombarded by variety of stimuli at the same time has no way but to process only what is relevant and important. Attention is therefore a fundamental cognitive function which has been extensively studied in non-human primates but much less in non-primates such as rodents (Chudasama and Robbins, 2004; Dalley et al., 2004; Robbins, 2002) and chickens (Sridharan et al., 2014). The main reason for this is that despite the powerful experimental possibilities that exist in particular for mice, we are lacking efficient behavioral paradigms that probe attention in mice during processing of more complex (e.g. multimodal) stimuli (Buccafusco, 2009; Sahgal, 1993).

An important study on mice (Wimmer et al., 2015) has recently explored the top-down attention in sensory selection in a bimodal attention task. This behavioral paradigm, although difficult to achieve in mice, is a promising start for further exploration of the circuits involved in attention.

One of the major bottlenecks for achieving complex tasks is the training time. This factor has probably so far precluded an in-depth investigation of complex behaviors in mice by the broad scientific community. Automated training is a promising alternative to manual approaches since it can drastically reduce the experimenter time and increase the efficiency of training for complex tasks (Schaefer and Claridge-Chang, 2012). If the cost of the system is reasonable, this approach

moreover offers the possibility of running experiments in parallel and thus to investigate several alternative training strategies quickly. In addition, it can alleviate other drawbacks of manual training such as behavioral variability caused by handling stress, imposing the time of day and duration of the training on the animals and the need for water restriction. This approach also has a good potential to make behavioral results more comparable between different laboratories since the hard- and software as well as the training protocols are easily shared, and contact of the subjects with the experimenter that can induce variability is minimized. Finally, automated training also has important advantages for animal welfare, including social housing in an enriched environment, stress reduction by minimizing water restriction and contact with the experimenter, and reduction of the number of animals required for a given experiment (Richardson, 2012).

4.1 Background of Automated Training

Automation is not a new concept (Guarnieri, 2010). Thanks to many recent advancements in chipsets and micro-controllers, the field of Neuroscience has also managed to use these technologies for quick and efficient feedback systems. Behavioral/physiological readouts now can be used to give almost instantaneous feedback to the system under investigation. For example, not long ago people had to trigger the auditory stimulus for fear conditioning by pressing the play button of a cd-player, but now stimulus onset can be bound to realtime spike detection algorithms processing 1000 channels simultaneously in a closed-loop feedback system (Jun et al., 2017).

In my review of the literature on the topic I found two major sets of studies: 1-Passive: systems that use tracking methods such as video tracking, RFID (radio frequency identification), infrared (or a combination of these) for assessing the individual activity level and/or social interactions of the animals. 2-Active: Studies which provide feedback to the animals based on their behavior, and can thereby perform the training of the animal without direct interaction with the experimenter, e.g. in the home cage.

However, there are also studies which use the word automation to refer to more incremental improvements in their procedures – i.e. partial replacement/reduction of human labor with tools (He et al., 2015).

4.1.1. Passive Tracking

These methods are well suited for assessing the welfare and behavioral state (e.g. anxiety) of animals by measuring their social interactions or level of activity as well as comparing phenotypes of animal mutations.

Infrared beams are the least expensive (although imprecise) method for tracking the passage of animals between different locations. In one study design, they used simple infrared beams located on passages of a tripartitioned cage to measure the time spent in each section of the cage, while one of the partitions included a smaller cage containing another mouse. They used this setup to compare the "sociability score" of the animals toward another familiar or unfamiliar mouse (Nadler et al., 2004). In another study the commercially available "Labmaster" system was used for phenotyping a mouse model of spinocerebellar ataxia type 17 (Portal et al., 2013). This system uses infrared beams to score the activity of mice in the cage area.

Video cameras have been around for long time but the progress in quality of the images as well as the development of computer vision algorithms have made it now possible and economically viable to use them in experiments which need to record and assess behavior of animals over long times and/or in real time.

However since the laboratory animals look very similar, one challenge to video tracking methods is to mark individuals in socially housing conditions. In one study researchers used color-coded collars for monkeys for a real-time multi-camera 3d tracking system (Ballesta et al., 2014). In another study they used video cameras with RFIDs in a social and enriched environment to assess the behavioral phenotyping of mice as well as pair interactions and social dynamics (Weissbrod et al., 2013). Bains et al. used a combination of video tracking and RFID chips to track the activity of mice during day and night in a social cage. They argue that such a system can be used for assessing the activity of different strains and it gives a far deeper understanding than the conventional out-of-cage phenotyping (Bains et al., 2018). They also briefly compare the commercially available home cage monitoring systems (Table 1).

In addition, video tracking can be used for single or paired housing without a need for an identification method. In one study they recorded the activity of mice over a short period (20 minutes) and checked for the effects of analgesics on the behavior of the animals using a behavior recognition software called 'Home Cage Scan' (Roughan et al., 2009). In another study, using video tracking, they assessed and classified pairwise mouse social behavior such as following (chasing), going above each other, postures like nose to nose or nose to genitals, etc (Giancardo et al., 2013).

Table 1. Comparison of home cage monitoring systems. Reproduced from (Bains et al., 2018). References studies related to every system are available in the original paper.

System & Company/Institution	Camera	Strengths	Limitations
Home Cage Environment CleverSys Inc	Side	Detailed assessment of animal behaviors in home cage.	Solitary housing. No direct measurement of activity
GroupHousedScan CleverSys Inc	Top and Side	Detailed assessment of animal behaviors and social interactions in home cage.	Maximum of two individuals. Not compatible with standard vivarium
PhenoRack ViewPoint Life Sciences	Side	Compatible with High density IVC racks. Quantitatively measures activity and Automatically annotates rearing, drinking and feeding in home cage.	Solitary housing.
SCORHE NIH	Front and Rear	Compatible with standard high density IVC rack. Measures activity in home cage.	Solitary housing. Currently limited to black mice.
PhenoTyper Noldus Information Technology	Top	Can be multiplexed with drug delivery systems and operant systems to run bespoke experiments	Solitary housing in bespoke environment. Top view camera cannot track detailed behaviors.
ANY-Maze Cage Global Biotech Inc; Stoelting Co.	Top	Can be used in combination with weight transducers for measuring food and water consumption. Can measure running wheel activity and immobility defined sleep	Solitary housing. Mice need to be removed to bespoke environment.
Home Cage Analysis System Actual Analytics	Side	Mice remain in their home cage within established social groups. Social interactions Compatible with standard high. density IVC racks Videos overlaid with RFID identities for each mouse.	Takes two IVC rack spaces. RFID microchips inserted into mouse groin under general anesthesia.
LABORAS Metris, B.V.	N/A	Registers behavioral signatures for fine movements like grooming, eating and drinking.	Solitary housing. No video output. Mice need to be removed into bespoke environment.
IntelliCage NewBehaviour TSE Systems	N/A	Group housing. Possible to design custom cages set ups to run bespoke experiments.	No video output therefore detailed behaviors not recorded. Animals need to be acclimatised to the bespoke environment.
PhenoMaster LabMaster TSE Systems	N/A	Multiplexed with weight transducers and beam break detectors for measuring food and water intake along with activity measurements. Indirect gas calorimetry. Can be multiplexed with other equipment to run memory and learning tasks.	Solitary housing. Mice need to be removed to bespoke environment

4.1.2. Active Automation

These automation methods include systems which give feedback (stimuli, reward, etc) to the animals on the basis of the behavioral/physiological readouts done by sensors, video analysis, electrodes, etc. These approaches are ideally suited for operant conditioning paradigms. Animals can be housed individually or in groups. Group housing systems have the advantages of using only one training chamber for all the animals in the group (and are therefore cost effective) and enrichmening of the animal housing conditions through social interactions. However, these systems typically require some identification method (e.g. RFID) so that training for individual animals can be adjusted by the control system and the experimenter.

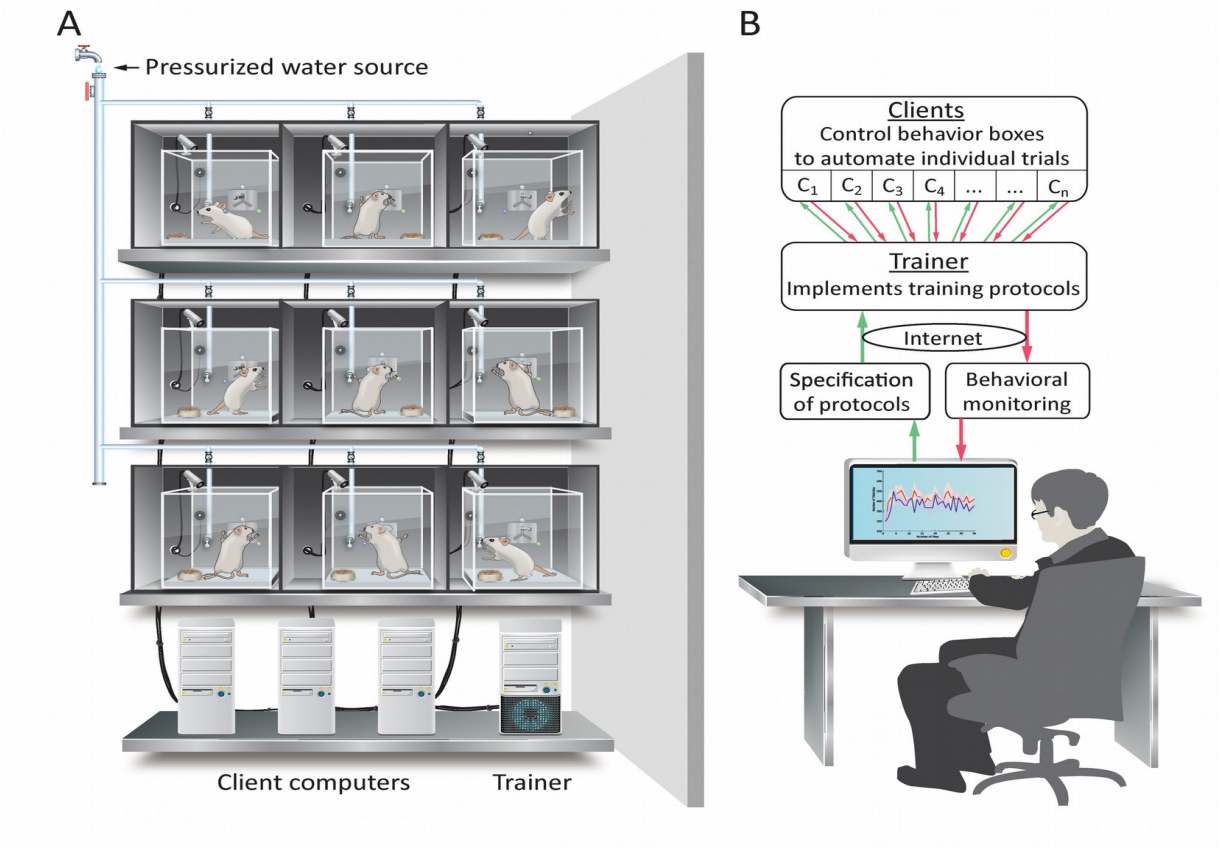

Figure 1. ARTS: A system developed in the Ölveczky lab for training rats simultaneously (Poddar et al., 2013)

Individually Housing Systems

In one study they reinforced individually housed mice in an operant conditioning task based on the mouse's location in the cage, which was monitored by video camera. The reinforcement was provided only during the dark phase in session lengths of 15 minutes and intervals of 1 hour. They used this system to compare the behavior patterns of mouse strains (Remmelink et al., 2015). Since 2013, the Ölveczky lab has contributed two interesting developments in this area. In one, they developed a fully automated system for simultaneously training several individually housed rats in which animals learnt a sequential lever pressing task to receive water reward (Figure 1). The training sessions were scheduled during the -rat's subjective- night and the start of the session was signaled by a tone and few drops of water. They named this system ARTS (Automated Training System for Rodents) and argued that it has good potential for acquiring more complex tasks and hence increasing the advantages of using rodents for studying neural mechanisms of complex behavior (Poddar et al., 2013). In another study in 2016 they presented a novel setup for long-term continuous electrophysiology recordings of neural activity as well as behavioral readouts for freely moving rats inside the home cage (Dhawale et al., 2016). An important aspect of the last study was a spike sorting tool which could help keep track of the recorded units over several days. One interesting further development from these two studies would be to combine the two approaches in order to follow changes in neural activity during the learning progress of the animal. However recording from the same neurons over long time periods during which the animal is freely moving is still a challenge for these studies.

In another report, scientists attached a single mouse home cage to a 5-choice serial reaction time task (5-CSRTT) operant chamber, so that the animals could learn the task self paced around the clock. They call this system CombiCage and the results indicate that the mice can acquire the task in 1 week using this system, while the conventional manual training takes, on average, 51.5 days (Remmelink et al., 2017).

Group Housing Systems

Group housing offers the advantage of cost effectiveness since a single training station can be used by multiple animals, along with providing animals with a social environment. One simple solution for this paradigm is to place the operant conditioning apparatus inside the home cage. Francis and Kanold (2017) placed a go no-go lever press apparatus inside the home cage of the mice (without individual identification), so the animals could perform the task ad libitum. Animals in isolation and in groups both learnt to perform a sound discrimination task. The advantage of this approach is that

it doesn't require a tagging system, while the downside is that individual training schedules are not possible, and the results can also be more variable due to presence of the other animals while an animal is performing the task.

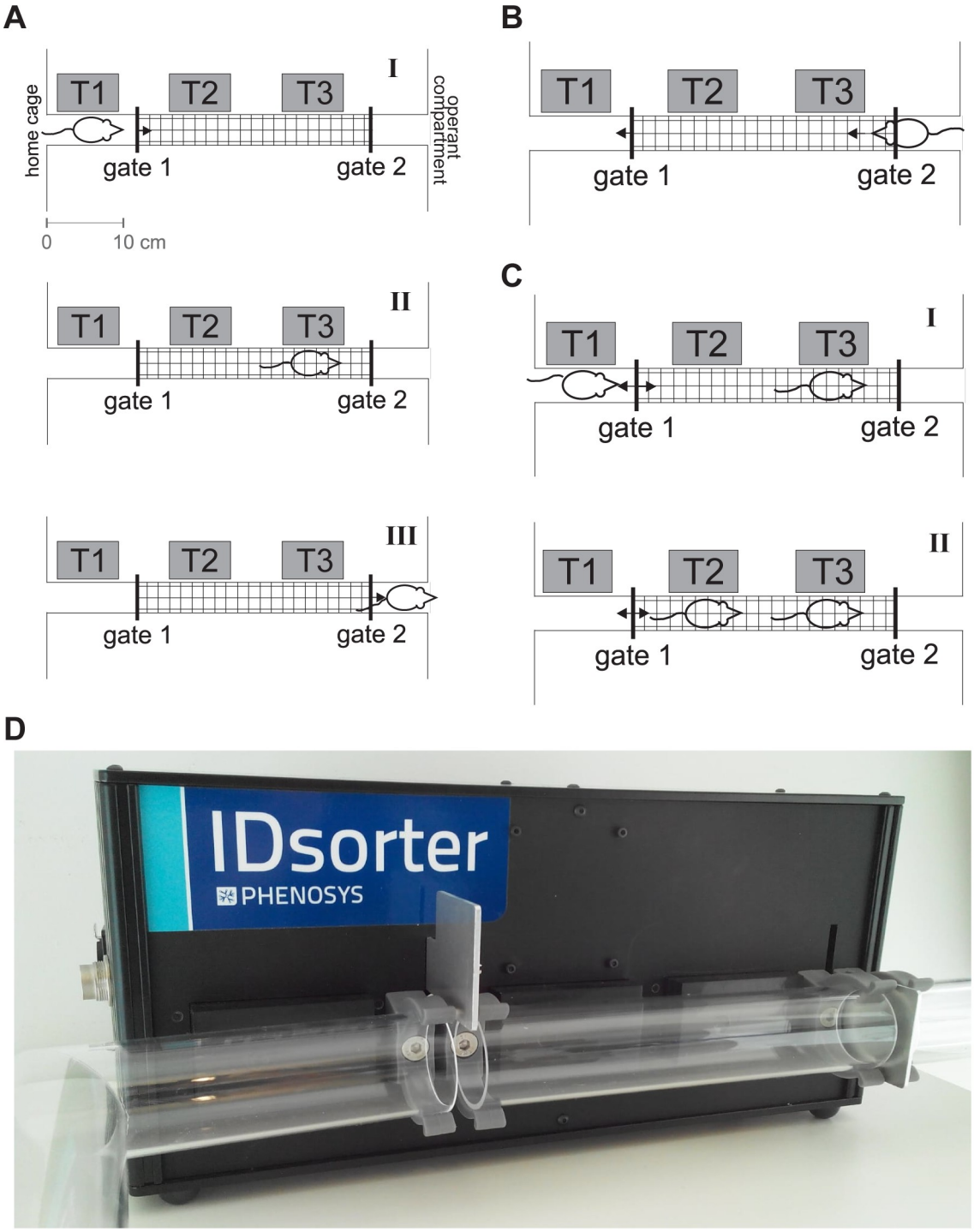

Figure 2. (caption on the next page →)

RFID antennas are useful for this paradigm because they can provide reliable identification to the automation system and they are minimally invasive (just an injection of the transponder, usually subcutaneously between the scapula). For example, scientists connected an operant conditioning chamber with touch screen to a cage of group-housed rats via an RFID gating system (called ID-Sorter, Figure 2) to train the animals for a trial-unique non-matching to location (TUNL) task. The animals learnt to pass through the identification unit and initiate the trials. The authors also compared the performance in automation with that of rats doing the same task in a non-automated setting, and found no difference (Rivalan et al., 2017).

Open source/free software and hardware are becoming available to help scientists in generating rapid, simple and cheap solutions for tool development. For example, there is a cheap solution for automated weighting of mice in their home cage using Raspberry Pi (Noorshams et al., 2017). In another study one group developed an automated setup for a social home cage in which the mice learnt to self-initiate a head-fixation to earn water. Automatic functional imaging was performed during the head-fixed period. They also published the blueprints and the code for this inexpensive setup; the control hardware is also a Raspberry Pi (Murphy et al., 2016). These two methods can potentially reduce the induced stress of weighting and head-fixation. Moreover since the head-fixations are voluntary it may increase the number of trials performed during the day in a cognitive task-such as a go/no-go paradigm. However an important open question is whether animals still perform similarly well when they are put into a manual training context, which is required in most cases for neural recordings, imaging and optogenetics.

→ (caption from previous page) Figure 2. The ID-Sorter. (A) The process of “sorting in”. (B) The process for “sorting out” (C) An unsuccessful sort due to presence of a second animal in the tube. (D) A photo of the device. The arrows indicate that the door is open and they point to the direction that the animal is supposed to go. The roman numbers (I, II & III) are the sorting steps. (sorting process schema adapted with modification from (Winter and Schaefers, 2011)

5. Aims and Objectives

The aim of this project is to develop a low cost, automated system for control of behavior in mice, in which mice can be trained efficiently on relatively complex tasks – in particular a bimodal attention task using audiovisual stimuli. One of my aims regarding the design of the system was reliability and flexibility. I needed a system that would operate around the clock for many weeks without interruption by potential glitches due to systemic or environmental events, such as for instance network failure or any form of animal behavior. To achieve this goal, I established an integrated set of pieces of software which allows me to plan a full training protocol (step by step shaping stages) by setting it's parameters, and let the animals learn the task by graduating automatically through the stages until they reach the desired behavior – with minimal interference of and time demands on the experimenter. In addition, I required a pipeline for data processing that gives me the ability to monitor the experiment outcomes online. Moreover, it is crucial that additional snippets of code can be incorporated with minimal effort, such as for calculating different behavioral outcomes that subsequently can be used as graduation criteria (e.g. performance over the last n trials, average reaction time, or amount of consumed water over the last day). The training program should also have the flexibility to be adjusted by the experimenter on the run, to streamline the behavioral shaping process.

In order to achieve the training of the bimodal attention task, I first tested the possibility of training the mice for simple tasks while validating the usability of the automated system regarding the welfare of the animals and their engagement in the training program. The first, simple task was a two alternative forced choice task with LED light on the ports as visual stimuli. In this manner I gained enough insight into behavior under automated settings to move to more complex tasks. Then I moved on to training the same task with more complex stimuli like frequency-modulated sweeps (auditory) and moving gratings (visual). I aimed to test the transferability of this method to experiments addressing biological questions (optogenetics or recordings), to demonstrate that the trained animals in this system will be able to perform under these experimental conditions. Finally, I incorporated the acquired knowledge to train the animals on a bimodal attention task.

6. Methods

6.1. Animals

Batches of 5-8 male wild type (C57BL/6J) mice were injected with RFID transponders and co-housed in an enriched cage of size Eurostandard TYPE III. Injections were done under brief isoflurane anesthesia in order to reduce anxiety from injections. The transponders were injected subcutaneously between the scapula. I only used siblings that had been co-housed throughout their life to minimize fighting. All animal procedures were executed in accordance with institutional guidelines, and approved by the prescribed authorities (Regierungspräsidium Darmstadt).

6.2. Apparatus

The social cage (Figure 3.A) is connected to the training station by a tube with two gates and three RFID readers controlling the passage of the animal into the training station (ID-Sorter, Phenosys GmbH, see Figure 2 for the sorting procedure).

The training station has three response ports (Sanworks) equipped with an infrared beam break to detect poking, and water reward delivery via a solenoid valve. The ports are controlled through an Arduino state machine (Sanworks' Bpod). Sound stimuli were presented with Psychtoolbox package for Octave through a pair of TDT MF1 closed field speakers via an Asus Xonar sound card. The visual grating stimulus is produced by Psychtoolbox and presented on a 7.0" monitor (BON FM-073SCH model). The simple visual stimulus is a white LED inside the response ports

6.3. Light Control

To control the light in the behavior chamber, I used another open source module called Blinkstick, which can control several LED-stripes at the same time using a diverse set of libraries for different languages. The module uses pulse width modulation (PWM) to adjust the brightness of the LEDs. I used 3 LED stripes of warm white color; two at the (out) sides and one on top of the training box. The surrounding light during the task performance was ~5 lx and during the time-out punishment and kick out protocol it was ~130 lx (measured by ELVOS LM-1010 Luxmesser).

6.4. Production and presentation of audiovisual stimuli

The frequency-modulated sweeps were produced by an online tool called wavTones. I used an in-house script to merge several sweeps and intervals of the stimulus depending on the experiment. The visual gratings were produced with Psychtoolbox, which offers a rich set of libraries for auditory and visual stimuli. Sound stimuli in the optogenetics group were up-sweeps of 5 to 15 kHz and down-weeps of 17 to 7 kHz; or pure tones of 7.5 and 12.5 kHz. In the rest of the experiments the sound stimuli were consecutive up-sweeps of 5 to 23 kHz and down-sweeps of 23 to 5 kHz. The length of the sweeps were 500 ms each. The moving gratings had spatial frequency of 0.04 cycles per degree visual angle and time frequency of 2 Hz, presented on the monitor 12 cm away from the location of the animal at the middle port.

6.5. Hardware and software architecture

The main components of this system are the ID-Sorter, sorter PC, server, Bpod, and the training chamber. (Figure 3). A custom-written server script programmed in python connects to the ID-Sorter to control the passage of animals. Another python script is used to run a port of Bpod software on Gnu Octave command line interface. This modular design powered with open source/free software on a Linux machine provided me with more robustness and stability while making debugging and innovations easier.

6.5.1. The Sorter and RFID technology

During a market overview, I found the ID-Sorter (Figure 2) by Phenosys as a solution that fits my needs. This sorting system is based on RFID tags and has been tested in literature (Rivalan et al., 2017; Winter and Schaefers, 2011). Through communication with the manufacturer I received a software update with which I could communicate with the device over a simple text TCP/IP protocol. The commands for this protocol are listed in table 2. The ID-Sorter uses a micro-controller to read the RFID antennas and to open/close the gates, and a TCP/IP protocol over the RJ-45 socket to send the information of read antennas and to receive the commands for opening and closing the gates. The sorter's PC can operate the sorter in two modes: 1-standalone mode and 2-network mode.

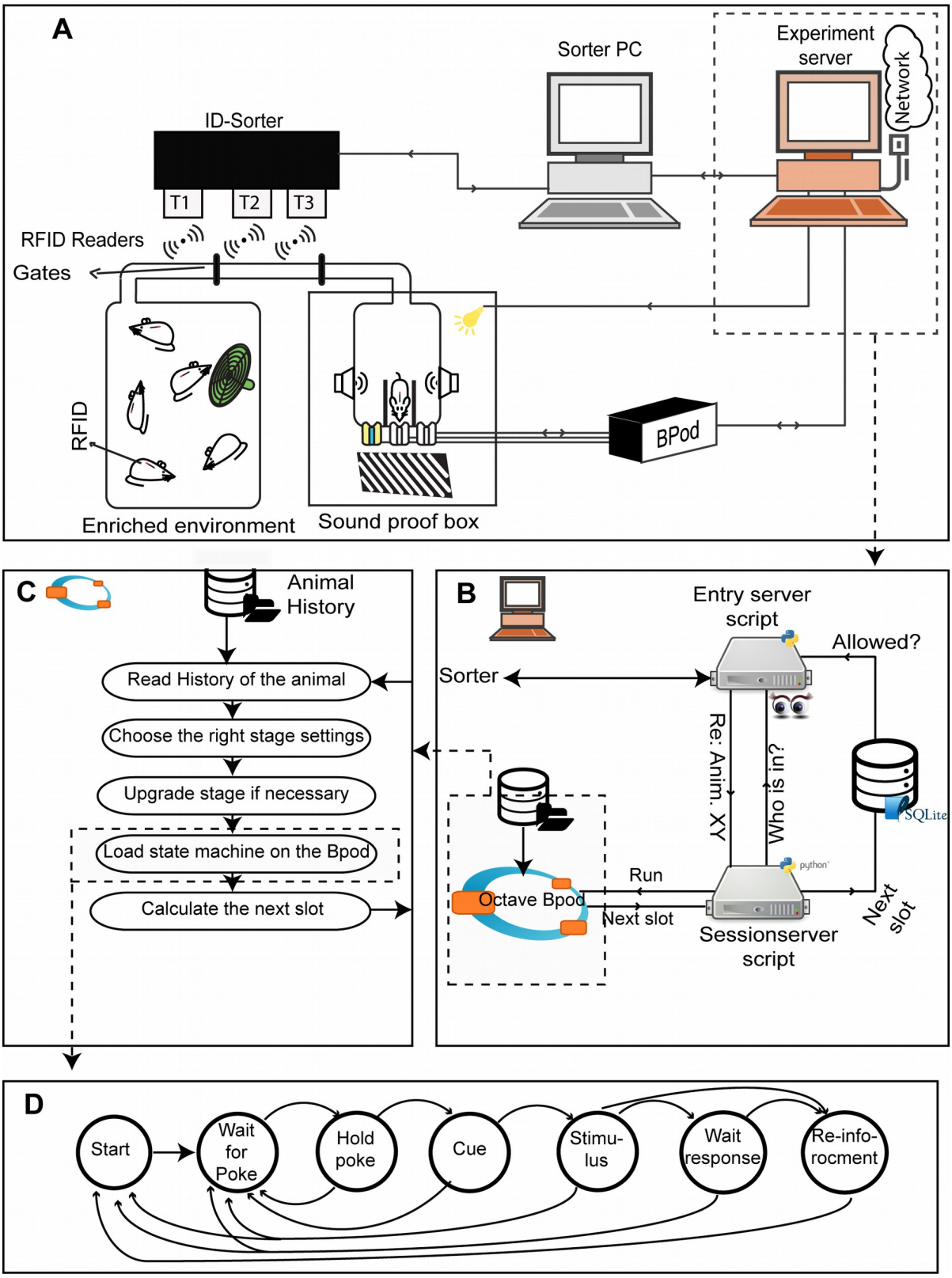

Figure 3. System architecture. (A) Hardware connections (B) The server (C) Behavior control hardware (Bpod) controlled through Octave. (D) The state machine structure of the protocol.

In the standalone mode, animals that their IDs are entered into a spreadsheet database file are "sorted in" by the sorter. There are parameters available to the user with which one can adjust the time to stay and frequencies of sorting for each animal. However this mode could only be useful for my pilot preparations, since there is no signal to the outside world that determines which animal is sorted in. This kind of signal is necessary for running experimental paradigms customized for each animal. The main use of this mode could be for indiscriminative training where it doesn't matter which animal is in the chamber.

The network mode operate using a simple TCP/IP text command protocol (Table 2). This protocol provided me with the required information (animal identity), allowed me to accept or deny entry to individual animals, as well as receiving information on errors and logs on my experiment server.

6.5.2 The Behavior Box

To run the behavioral task, I opted for an open source hardware design called Bpod. Bpod is a behavior measurement and control tool for rodent experiments. The open source quality made flexibility and further modifications possible. This means that all the information regarding the parts, including the controller board which is an Arduino micro-controller as well as the mouse ports are available to modify and produce in-house. The experiment protocol is loaded on Bpod through a usb connection in the form of a state machine. The states of the state machine have output functions, such as turning the port light on/off, opening the water valve, sending TTL signals, etc; as well as criteria for changing the state due to an event, such as a poke by the animal or a time out. The official release of Bpod software for running the experiments is written in MATLAB code, but since the accessible licenses for using MATLAB in the lab where network based (i.e. needed to be connected to the internet all the time), which could undermine the stability of the system, e.g. due to a network failure, I decided to adapt the software for use with GNU Octave. GNU Octave is a programming language for scientific computing which can also run MATLAB files. It is part of the GNU project, therefore it is a free software under the conditions and terms of GNU General Public license. In order to be able to use the Bpod software under Octave I needed to remove the Graphical user interface (GUI) entirely and to program a command line version of the Bpod software. This port is available as free software on Github (Mahyar Moghimi, 2017).

Table 2. Communication protocol with the ID-Sorter over TCP/IP.

Command	Direction	Description
start	PC->Control	Indication to RFID system to sort an individual into the cage
“in ind”	Control->PC	Request Individual with the name "ind" to be sorted in
• Response: “in yes ind”	PC->Control	Acknowledge in request, allow "ind" animal entry
• Response: “in no reason“	PC->Control	Acknowledge in request, deny animal "ind” entry, reason string follows
abort entry	Control->PC	Sent if PC previously sent "in yes in" but animal is not sorted in
• Response: "abort yes"	PC->Control	Sent if abort process successfully
• Response: "abort no reason”	PC->Control	Sent if the abort failed for any reason
“entry completed“	Control->PC	Sent if PC previously sent "in yes ind” and animal is sorted in
• Response: "entry yes"	PC->Control	Sent if entry was processed successfully
• Response: "entry no reason"	PC->Control	Sent if entry failed for any reason
abort run	Control->PC	Sent if Control needs to stop schedule execution for any reason
• Response: "abort yes	PC->Control	Sent if abort process successfully
• Response: "abort no reason"	PC->Control	Sent if the abort failed for any reason or the command was invalid
end reason	PC->Control	Individual should be sorted out, reason string follows, generally due to successful schedule completion
out ind	Control->PC	Individual "inO" has been sorted out of cage
• Response: "Out yes ind"	PC->Control	Confirms out was processed for "ind"
• Response: "Out no reason"	PC->Control	Indicates reason why "ind" was not sorted out, most likely due to bad command or invalid operant ID
session active	Control->PC	Heartbeat command
• Response: "session no reason"	PC->Control	Only occurs if the command contained invalid characters
• Response: "yes"	PC->Control	Successful Heartbeat response

6.5.3. The server

The experiment server is the main component to orchestrate the whole experiment. It has two main server scripts that run in parallel and exchange information: The “Entry server” which controls the sorting procedure of the animals (communicating with the ID-Sorter) and the “Session server” which is responsible for running the training session for the sorted animal.

The entry server first receives the command (table 2) from the sorter which indicates an animal is asking to be “sorted in”, in the form of “in ind” where “ind” is the id/name of the animal. The server checks the database for that animal to see if it is allowed to be sorted in and whether the time for the next slot of the animal has arrived. Through this database I can disallow individual animals from using the system or set a time for their next slot. To develop the database application I used SQLite which is a lightweight and file based SQL database. The entry server then sends the corresponding command to the sorter’s PC whether to sort the animal in or not. This command can be “in yes ind” or “in no ind”.

The “Session server” runs a continuous loop in which it asks the “Entry server” which animal is currently sorted in, with a simple text command over TCP/IP protocol in this form: “whoisin”. The entry server in response would send back the name of the animal (“ind”) or stating that no animal is sorted by sending: “noone”. When the name of the animal changes, the session server runs a library for running GNU Octave code inside python (called oct2py) to run the actual protocol for that animal through the command line version of the Bpod server. After the session is finished, the session server receives the next slot for the animal and writes it to the database. Both scripts were programmed in Python language because of it’s quick development time and availability of libraries (especially for running Octave, TCP/IP and SQLite).

6.5.4. The Protocol Design

In order to make all data and debugging consistent, I decided to use a single file protocol. I designed one protocol (i.e. the state machine) which runs with different parameters depending on the stage which the animal is in. I also adopted the “Agile software development” strategy which means that without having a grand design, I first came up with a minimal working software and had a continual improvement as I was going through new changes and needs of the projects. The protocol code is modular but it has now expanded to more than 800 lines which makes it’s direct reuse and adaptability difficult for other projects. However I believe that, when the concepts and ideas are

understood, coding a new protocol can be quite fast. The main modules of the protocol are shown in Figure 3C.

6.5.5. The State Machine

The protocol that is loaded onto the Bpod has the characteristics of a state machine model (Moore, 1964). At every time point during running, the machine will be in one of it's finite number of states. Every state has a timer and can have several output functions as well as transitions. The output functions are the effects of the machine on the external world. For example in my system, during one state, the LED light on one/several ports can be turned on, a trigger can be sent to one of the digital outputs and/or a soft code can be sent back to the governing computer which will in turn trigger an auditory stimulus. The transitions list (or: state change conditions), for every state, is a list of events and the accompanying next state for that event. For example, when the animal breaks the infrared beam on one of the ports (event) while the stimulus is being played (the "stimulus" state) the system will go to the reinforcement state (next state). It should be noted that when the timer of the state runs out, an event called "Tup" is triggered which will be handled by the corresponding transition function for this event. The major states of my protocol design are shown in Figure 3D.

6.6. Viral injections and Optogenetics

Mice were anesthetized using 1.5% Isoflurane and placed on a stereotactic frame. They were injected by AAV2/5-CamKII-ArchT-GFP in the A1 area bilaterally (stereotactic location from Bregma: M-L: ±4.6 and A-P: 2.6, 650 µm below the surface of the Pia matter)

I used a yellow laser with λ= 529 nm and intensity of ~19±1 mW optical output power measured (measured by THORLABS PM100D) at the end of the fiber connecting to the implants. In sessions with laser, 15% of the trials were randomly assigned to be delivered with laser during the stimulus presentation. The laser was "on" from 100ms before the sound stimulus play starts until the end of the stimulus.

6.7. Histology

The mice that were previously injected with ArchT were euthanized and transcardially perfused with 4% paraformaldehyde (PFA). The brains were dissected and kept overnight in PFA in 4°C and then kept in Phosphate-buffered saline (PBS) solution until the slicing time. The brain tissues were sliced using a vibratome (LEICA VT 1000s). The slices were mounted on slides and imaged using a Zeiss AxioZoom microscope.

6.8. Statistics

The number of experimental recordings and animals used in each experiment is indicated in the Figure legends. Statistical tests were performed using Matlab, p-values and statistical tests used are indicated in the Figure legends. Data were first subjected to a Shapiro-Wilk test of normality, and based on the result to the indicated parametric and non-parametric tests.

6.9. Troubleshooting and Exclusion Criterial

Animals that lost their transponder during the experiment were excluded from the experiment and analysis. Also one batch of animals was stopped after few days of experiment because of too many interfering issues.

7. Results

7.1. The Automated System

In order to test whether the concept of automation can address the envisaged issues, I first tested a batch of animals on a simple task. I wanted to make sure that the animals can use this system safely, get enough water and engage in the task. All animals could reach the training station on the first or second day. Every day the amount of delivered water was calculated by the software and in cases that an animal didn't receive a minimum of 1 ml water, extra water was delivered in a separate cage for free. Since there was no method available to ensure that animals are consuming the water being delivered through the valves, and for animal welfare reasons, I additionally weighed them manually every day to make sure they are keeping their healthy weight.

In the first batches, I applied a free access strategy. The animals could go back into the training chamber immediately and start a new session. In this case I noticed that some animals tend to go in very often; almost immediately after the end of their session. Since this could prevent others from freely accessing the training chamber, I added a criterion of non-consecutive entry, in which an animal could enter the training chamber only after (at least) one other animal has performed a session. I later developed the system to keep track of time slots for animals. In this system a new session can only start after two hours have passed from the previous session. Moreover if an animal is performing badly (not getting engaged in the task) and is still getting more than their daily allowance of water, the system can adjust accordingly.

In free access batches, depending on the difficulty of the training stage, the animals usually received more than 1ml of water per day through the task (Figure 4.B, top), which is their minimum daily allowance. Their weights were fluctuating between 85% up to 120% of the initial body weight (Figure 4.B, bottom).

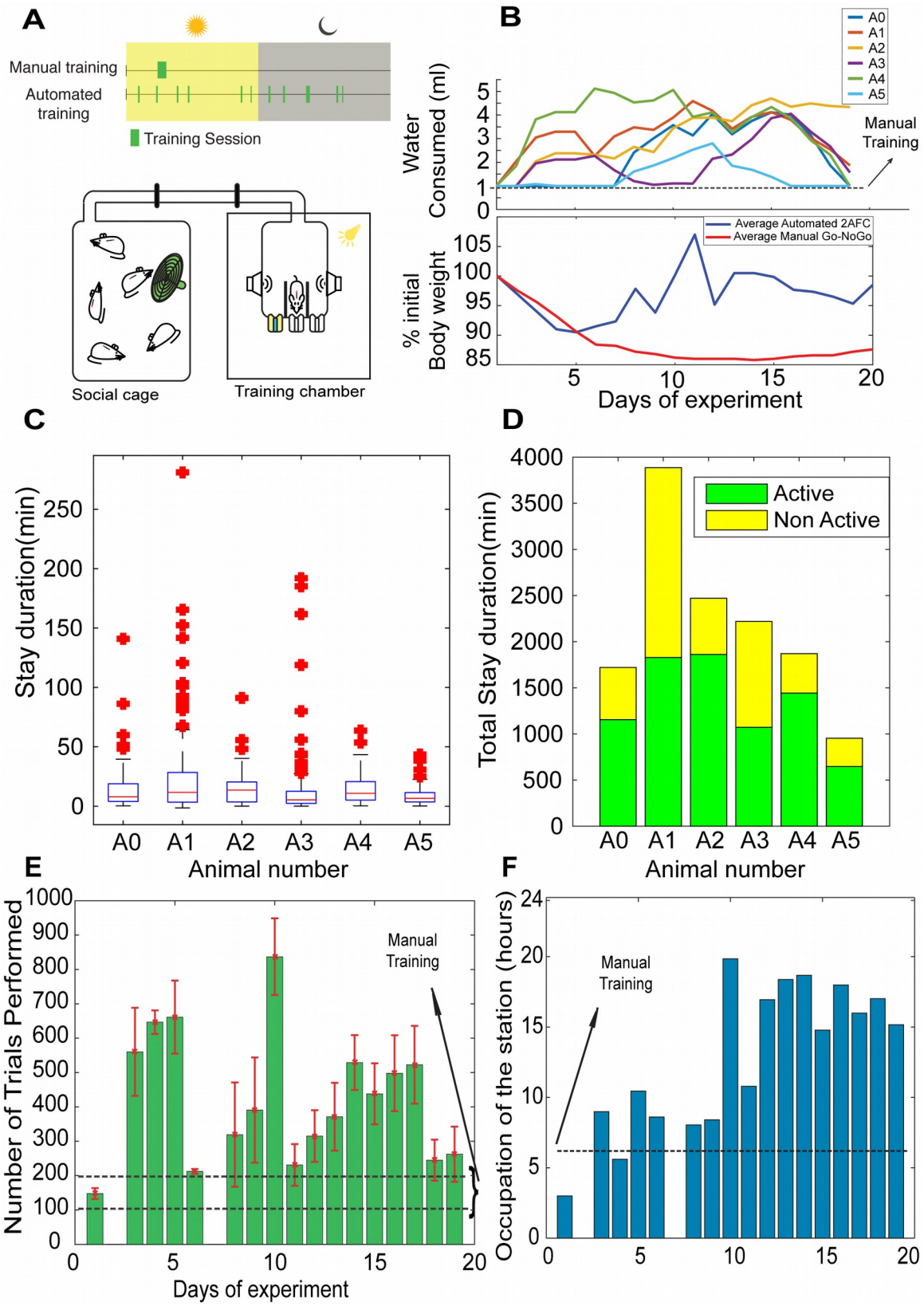

Figure 4 (caption on the next page →)

The training station was occupied most of the time (Figure 4.F). However the animals were not active all the time in the training station (Figure 4.D). For example, especially for the batches without restrictions on their entry, sometimes animals were staying in the training chamber long even after their session was over (Figure 4.C), or they were going to the chamber but not getting engaged in the task. The length of the session was 20 minutes minimum but if the animal showed activity (poking in any port) the system continued offering trials to the animal, until the animal was inactive for a 2 minutes period. I later came up with strategies to address these issues. However the active time (Figure 4.D) and number of trials performed (Figure 4.E) were still far in excess of manual training strategies.

In the first two batches I noticed that sometimes animals go into the training chamber but they don't engage in the task or they stay long after the session is finished (the yellow area in the bars of Figure 4.D). In order to prevent the unnecessary occupation of the training chamber, I added a "kick out" protocol consisting of loud white noise accompanied by the house light to encourage the animals to go back to the social cage immediately after the end of the session. In rare cases that I observed that an animal was still staying in the chamber, I added a constant air-puff through all three ports to the "kick out" protocol, which was effective in encouraging the animals to leave the training chamber immediately.

→ (caption from previous page) Figure 4: Operationality of the automated training system and animal welfare. (A) Top: Schematic of the expected dispersion of sessions across a day of training in automated paradigm in comparison with manual training. Bottom: Schematic of my automated home-cage training system. (B) Top: The amount of water consumed over days of experiment for one representative batch of animals (data smoothed, note that 1ml of water per day was supplied to every animal independent of performance). Bottom: Changes in animal weights in the automated training system (blue) compared to mice manually trained in head-fixation (red). (C) Distribution of the duration of stays in the training chamber. (D) Total time spent in the chamber over the course of experiment by every animal. Active time: the time spent on the task, Non-active time: the time spent in the training chamber after the session is over(E) Average number of trials performed per day and comparison to manual training in head-fixation (the system was down for maintenance on days 2 and 7 of the experiment). (F) Occupation time of the training chamber over the course of experiment and comparison to manual training in head-fixation. The comparisons with manual training are from a Go-Nogo task performed by Leona Enke in the lab.

7.2. First Group: Simple Visual Task and Light as a Conditioned Reinforcer

The most simple task, similar to my final task, was a version of 2AFC task with light stimulus on the port. Also in my pilot experiments, I had noticed that the animals can easily learn the task with this simple visual stimulus (the LED light inside the port). The initial idea was to later use the visual stimulus as a conditioned reinforcer (Skinner, 1951) for further training the animals for the auditory task. In this regard I tried two cohorts: 1-Late auditory (n=9) and 2-early auditory(n=6). (Figure 5).

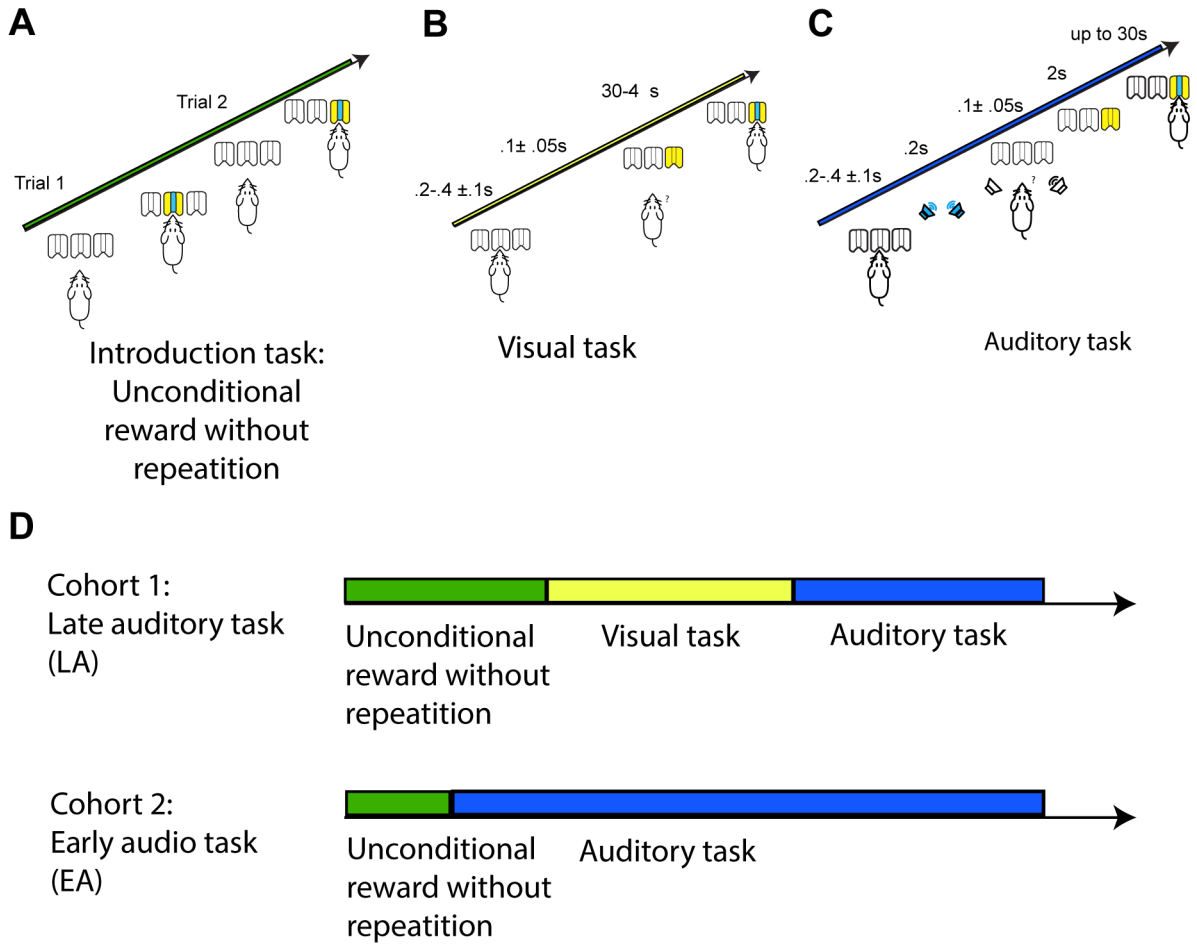

Figure 5. Task structure for the first group. Task structure for (A) unconditional reward without repetition stage, (B) the visual task and (C) the auditory task. (D) The series of tasks for the each cohort over the course of the experiment.

Both cohorts underwent a first introduction stage where they were receiving unconditional reward without repetition (Figure 5.A). In this stage the animal can poke in any of the three ports on the front wall of the chamber to receive a water reward preceded by the LED light on that port. The light remains on until the animal exits the port (i.e. stopped drinking). The reason for having the

light in this stage was to associate the light with reward as much as possible. This stage lasted for 100 to 200 trials, depending on the number of trials an animal was doing in each session.

7.2.1. Group 1, Cohort 1: Late Auditory Task With Conditioned Reinforcer

The first cohort of animals (n=9) were upgraded to the first stage of the 2AFC task with light (fig 5.B) after the unconditional reward without repetition stage. In this stage the animals were trained to respond in the port which was lighted up (on the right or left) after they initiated the trial by poking in the middle port and holding it for a short period of time. The time to hold the poke was increased from 200 milliseconds to 450 milliseconds over the course of the experiment by the experimenter manually. In the first stage the animal had the chance to correct it's first choice without the need to initiate the trial again. This option was cancelled after the animal showed an initial good performance (at least 70%). The animal was rewarded immediately after poking in the correct port.

As expected, learning to this light stimulus was quick (Figure 6 & 7). After the animals in the first cohort were expert in the visual task (at least 85% performance), I tried to use the visual signal to associate the auditory stimulus with the correct port. This means that the system played the sound stimulus after the trial initiation and then immediately turned on the LED light inside the port (Figure 5.C).

All animals in this cohort learnt the visual task to an expert performance of 85% and higher (Figure 7.A). Three out of nine in this cohort excelled in the auditory task, three animals showed initial learning of the auditory task and the other three either didn't learn the auditory task at all or were very slow in learning the visual task so that only at the last days of the experiment they just reached a high performance of the visual task (example performances in Figure 6).

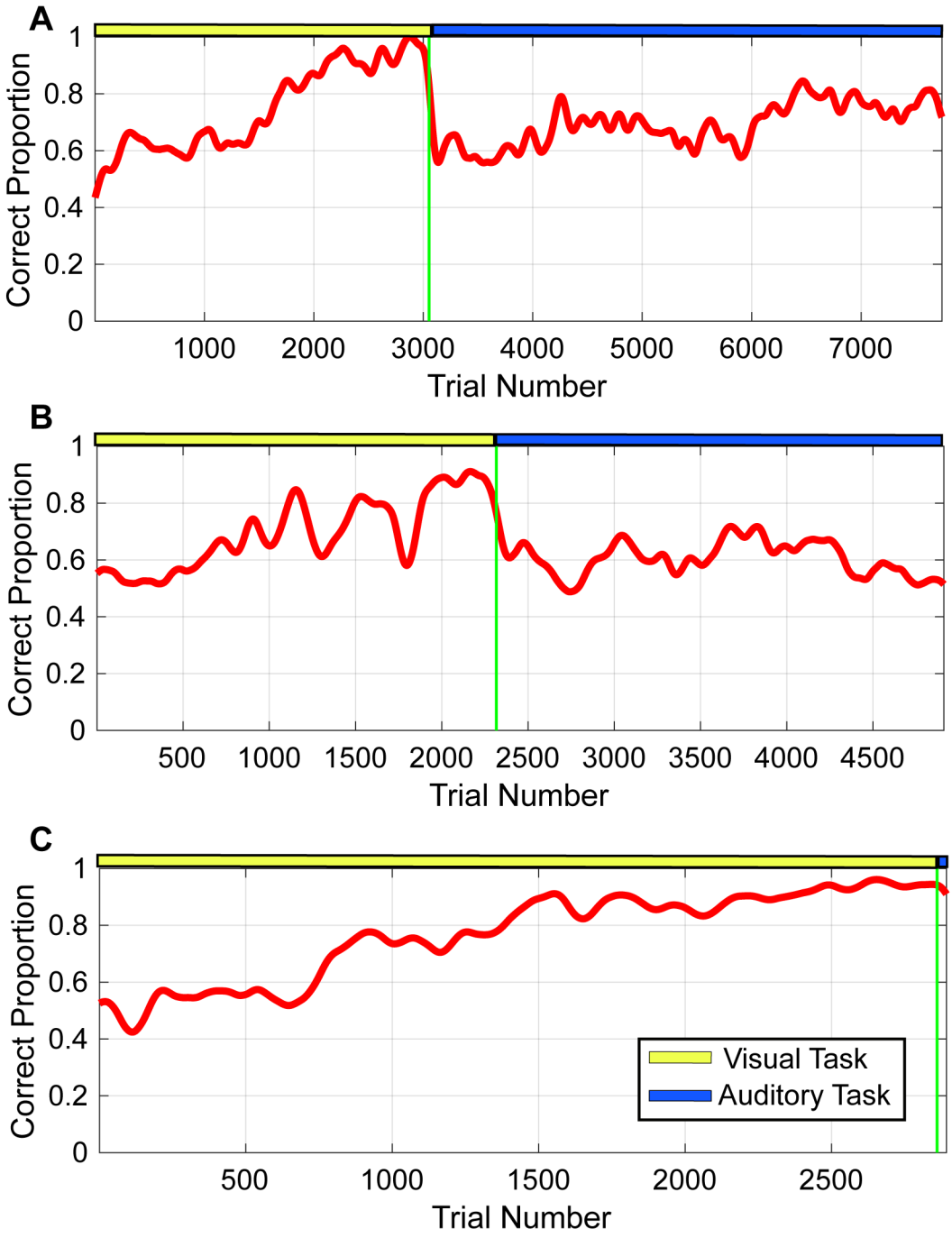

Figure 6. Example performance of animals of the first group (cohort 1) during the simple visual task with light as a conditioned reinforcer. (A) An animal that learned both visual (yellow bar) and the auditory task (blue bar). (B) An animal that only learned the visual task, but failed to acquire the auditory task. (C) An animal that learnt the visual task only at the end of the experiment. Data are presented as bins of 200 trials.

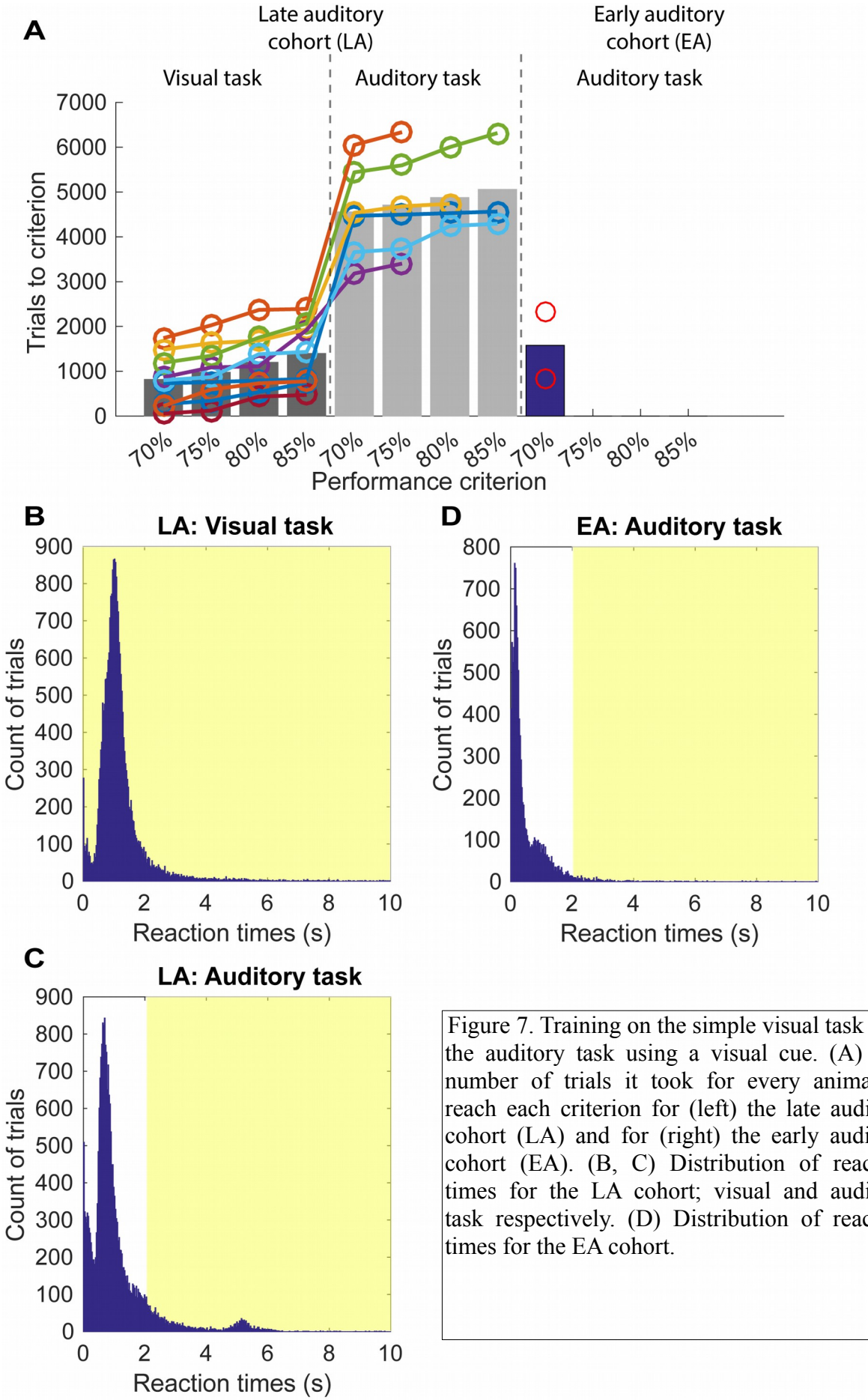

Figure 7. Training on the simple visual task and the auditory task using a visual cue. (A) The number of trials it took for every animal to reach each criterion for (left) the late auditory cohort (LA) and for (right) the early auditory cohort (EA). (B, C) Distribution of reaction times for the LA cohort; visual and auditory task respectively. (D) Distribution of reaction times for the EA cohort.

7.2.2. Group 1, Cohort 2: Early Auditory Task With Conditioned Reinforcer

The second cohort was introduced to the auditory task right after the introduction stage (see above). The trial structure and parameters were the same as for the late auditory cohort. This cohort barely showed any performance in discrimination of the auditory stimulus. While the intention was to use the light as a conditioned reinforcer, it should be noted that the mean reaction times (Figure 7.B, C & D) in both cohorts were less than 2 seconds. Therefore, the light was not perceived by the animals after the presentation of the auditory stimuli in most of the trials. I conclude that the light could not act as an effective conditioned reinforcer in this experiment design.

7.3. Group 2: Long Stimulus Time

Since the previous strategy of using light as a conditioned reinforcer did not work as expected, I decided to use another strategy which would directly confront the animals with the auditory stimulus. The problem with just playing the stimulus after the trial initiation is that the probability of the animal poking into the right or left port after initiation is low (in the first stage of training). Meanwhile if we increase the response window too long, the animal would be unlikely to associate the reward with the stimulus. Therefore I decided to repeat the stimulus until the animal received the reward.

This time I tested 19 animals (in 3 batches) in the automated setup for a 2AFC task, with a strategy similarly reported for acquisition of a bimodal task (Wimmer et al., 2015). In this training strategy, after the introduction stage, I repeated the stimulus for a relatively long time (30 seconds down to 2 seconds along the progression of learning stages) after the trial initiation, until the animal collected the reward (Figure 8.A). The animals were kept in this stage until they showed an initial performance in discrimination of the stimuli (at least 70%). The main reason for pursuing this strategy was that a pilot experiment with the previous non-learners showed promising results (data not presented in this report). I tried two cohorts: 1- Auditory and 2-Visual. The stimuli for the auditory cohort were consecutive frequency sweeps and the stimuli for the visual cohort were moving gratings on the monitor.

The auditory cohort showed a high discrimination performance. Two out of four mice reached 85% performance within less than 3000 trials (Figure 8.B). The visual cohort on the other hand did not even show preliminary discrimination. I tried to improve the task on the second batch by presenting half screen gratings in the first stage and on the third batch by graduating earlier from the first stage (30 sec stimuli with the permission to correct in case of a mistake) as soon as the animal showed

learning of the 2AFC task structure; i.e. initiating the trial and seeking the reward on right/left immediately afterwards. None of these animals showed a performance significantly higher than chance levels.

In preparation for the optogenetics group (next group), and in order to make the task easier, I ran another batch of mice with (at the time appearing to be) minor changes to the paradigm. I changed the stimulus to have intermittent silence in between sweeps and the poke holding time needed to start the trial was kept fixed (did not increase over stages). The stimulus included 500 ms sweeps of 7500 to 12500Hz with 500 ms silence in between, and the hold time needed to start the trial was kept fixed during all stages to 200ms.

No animal from this batch reached a performance above chance level after 19 days (~5600±950 trials). My primary interpretation for this result is that the variability and gradual increase (over stages) of the holding time demands the animal to listen to the sound in order to find out when to go for the right or left choice. Alternatively if the holding time is constant the animal may learn to go for the right or left port after a brief poke in the middle port, while ignoring the sound. Another possible consideration for this malperformance might be the intermittent stimulus which can be computationally more difficult to detect compared to the consecutive sweeps.

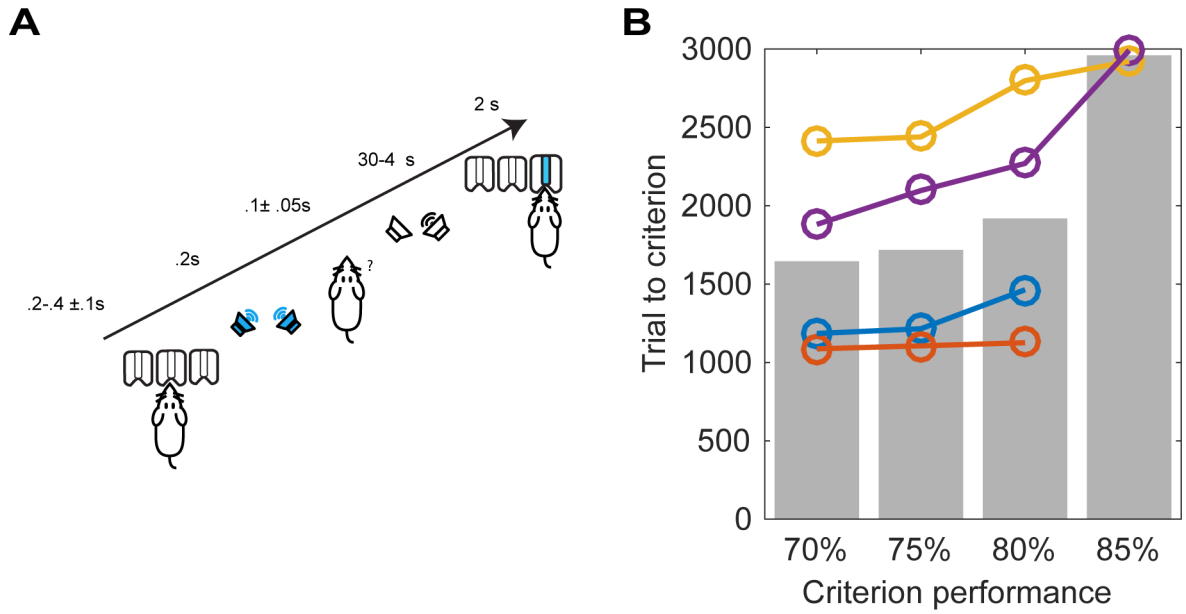

Figure 8. The approach of repeating the stimulus in the first stage. (A) The task structure for the auditory task in this approach. After the cue, the stimulus is repeated for a relatively long time until the animal pokes into the correct port (B) Trials to criterion for each animal (only the auditory cohort is shown here, the visual cohort with moving gratings performed at chance levels).

7.4. Group 3: Optogenetics

One of my concerns regarding the adoption of the automated home-cage training system was it's transferability to real world experiments addressing biological questions. The animals in the automated home-cage training system are being trained at their preferred time (mostly at night) and they have relatively short sessions but for an experiment for optogenetics inhibition/excitation, electrode recordings or imaging we will need longer sessions at the experimenter's work time (mostly during the day).

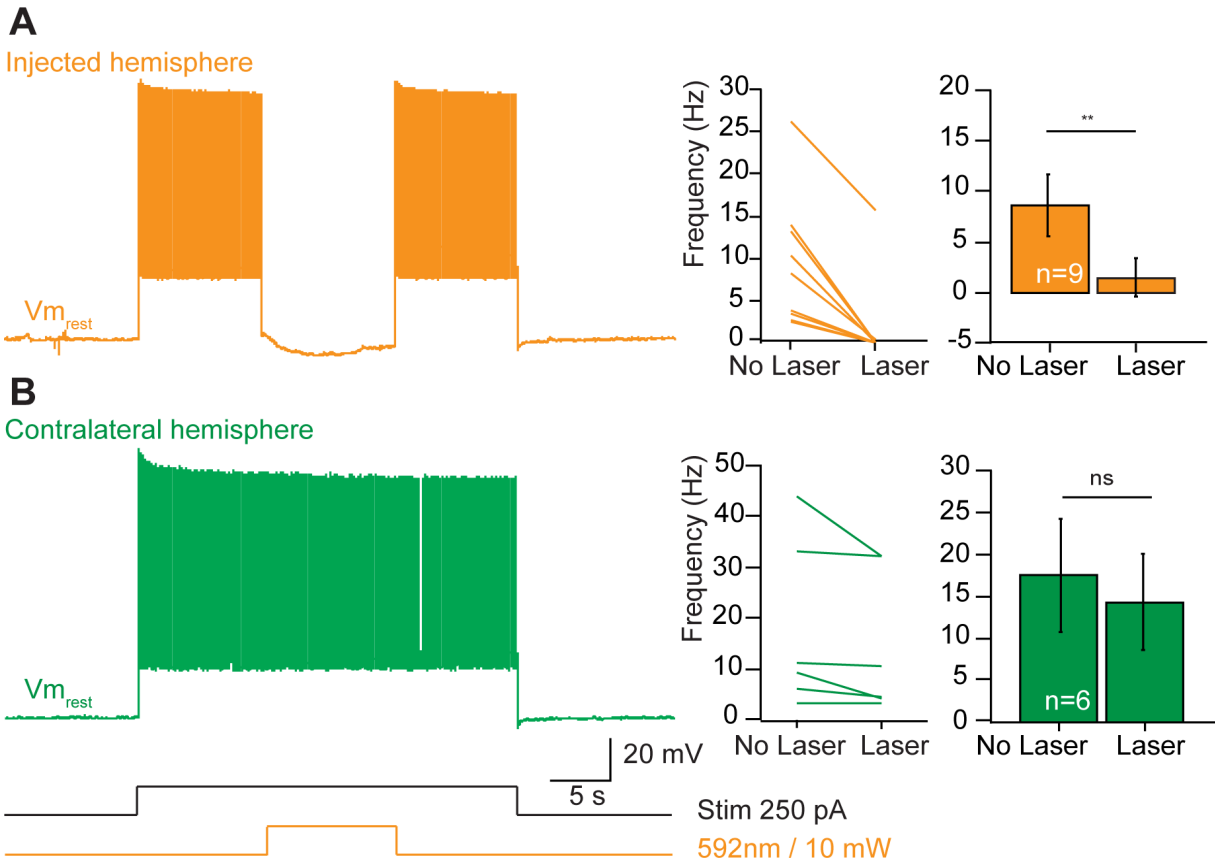

Figure 9. Validation of optognetic inhibition using ArchT in acute brain slices. (A) Left: Example response of a neuron to an injection of +250 pA current, and inhibition of firing by ArchT (laser application indicated at bottom of B). Right: the firing frequency of patched neurons during laser and without laser. (p= 0.002, Wilcoxon matched-pairs signed rank test). (B) Same as A but with recording on the contralateral hemisphere.(p= 0.0625). Data courtesy of Rogier Poorthuis.

7. Results

Previous patch clamping experiments in the lab (performed by Rogier Poorthuis) have shown that we can inhibit auditory cortex pyramidal neurons (CaMKIIa positive) by expression of ArchT (a H+ membrane pump, Chow et al., 2010; Han et al., 2011) and application of yellow (529 nm) laser (Figure 9). Based on this, another set of experiments in the lab (Dalmay et al, in preparation) has shown that the inhibition of auditory cortex during expression of fear memory results in a reduction of freezing for the animals who have associated the sweep stimuli to a mild foot shock, and less effect in animals that were conditioned to pure tones. This finding suggests that the auditory cortex is necessary for the recall of the memories of more complex stimuli like sweeps but it is not essential for the recall of simple stimuli like pure tones.

In this regard, we designed an experiment to test whether the auditory cortex is required for the animals to discriminate the sounds in a 2AFC task after learning. we injected 5 mice with an adeno-associated viral vector expressing ArchT and GFP in the auditory cortex, and trained them on a 2AFC task in two different cohorts using either frequency-modulated sweeps or pure tones as stimuli.

The animals were injected and implanted before the experiment. After reaching a high performance of 80-85%, the learner animals were then prevented from doing any trials in the automated system but they were still maintained in the same social cage. Instead, I performed one manual session per day with them, for the purpose of habituating the animals to the optogenetics settings, so that the animal was connected to the fiber but without using laser. This way I would ensure that I have a known baseline of behavioral performance before the laser for every animal.

The pure tones cohort learnt the task quickly compared to the sweeps cohort (Figure 10.B). Starting with the manual sessions, I faced a mechanical problem regarding the size of the cage and implants. The animals could not easily access the space between the separators on either side of the stimulus ports while the fibers were attached to the implants. Therefore I removed the separators so that the animals could move freely between the ports. This resulted in most animals developing unwanted behavior strategies. After a while they were responding to the sound too quickly (before the end of one sweep), and with performance at chance level. Placing these animals back in the automated schedule did not help them reach their previous high performance levels.

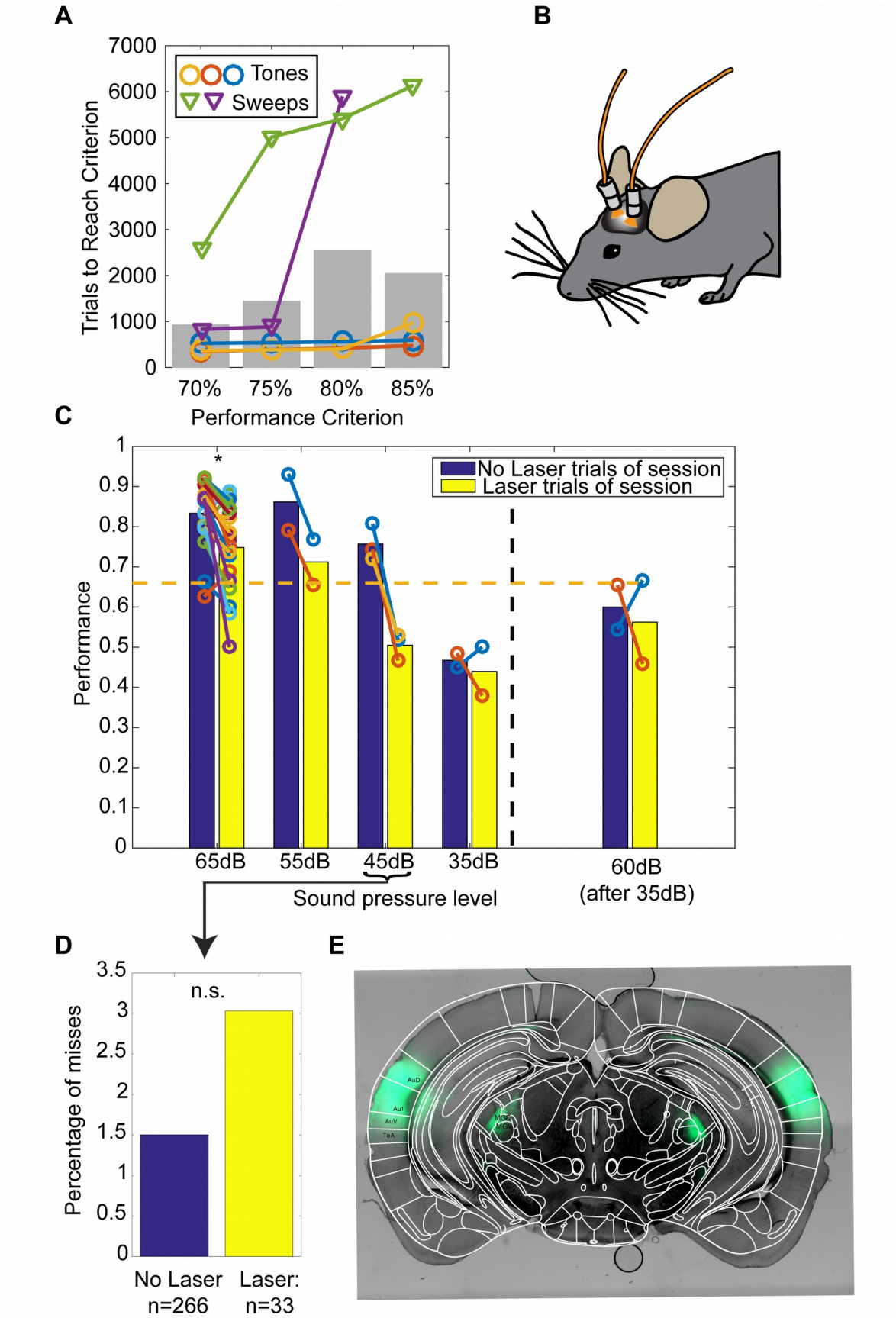

Figure 10 (caption on the next page →)

One of the animals in this group kept it's performance high in the optogenetics environment. It was also consistently performing the task above chance level in the laser condition (Figure 10.C). However the performance under laser was slightly lower than without the laser. I continued this condition for several days and the results stayed the same. After that I reduced the sound pressure level of the stimulus. Interestingly at 45 dBSPL the laser performance dropped to chance level while at 35 dBSPL both laser and non laser were at chance level.

Of course this is a small sample and far from a conclusion. However considering that 35 dBSPL is almost un-hearable (same level as the baseline noise level of the environment), it is clear from this data that the animal could not hear the 35 dBSPL stimuli. But it's success on 45 dBSPL and comparing it to the higher sound pressure levels suggest that the animal can perceive the sound at 45 dBSPL, albeit with less accuracy than for higher intensities. At this stage my preliminary hypothesis (which should be tested in further research with higher statistical power) is that recognizing a weak stimulus in a noisy environment requires processing (like a top down attention) which involves auditory cortex.

→ (caption from previous page) Figure 10. Optogenetic inhibition of auditory cortex during performance of the 2AFC task (A) Trials to criterion in ArchT-expressing animals for either frequency-modulated sweeps or tones as stimuli. (B) Schematic of a mouse with fiber and implants (image courtesy of Tamas Dalmay). (C) Performance of one animal during manual optogenetics experiments. Inhibition of auditory cortex causes a stronger behavioral deficit for stimuli with lower sound pressure levels. The horizontal dashed line is the maximum possible chance level if the animal adopts a strategy of win-stay-loose-change because of the correction trials. (MWW test, n=19 sessions, p=0.012) (D) Comparison of the number of misses in the 45 dB SPL condition. (Fisher's test, p=0.445) (E) ArchT-GFP expression in auditory cortex.

7.5. Group 4: Bimodal Attention Task

I trained 5 animals in alternating blocks of auditory and visual 2AFC task. The original plan was that when animals learn the task to a certain criterion in alternating blocks of auditory and visual trials, I start to add the other modality (signaling the opposite port) with low intensity as a distractor to every block and then after they start to perform well under the distractor, to increase the intensity of the distractor until both distracter and the stimulus are at their highest intensity. The type of the block was signaled to the animal through the cue. For the auditory blocks I used a 200 ms blue noise immediately after the initiation of the trial and for the visual blocks I used a 200 ms brown noise.

All animals in this group learnt the visual task much quicker than the auditory task (Figure 11.A light grey). At this point I noticed that they can obtain enough water by just performing the visual trials and ignoring the auditory task. In order to prevent them from doing so, I reduced the percentage of the dominant modality trials down to 10% of the trials of every session. This should ensure that the animal needs to get involved in the non-dominant modality while at the same time not losing the performance in the dominant modality. After the mouse started to perform the auditory task, I planned to place it back on the 50%-50% schedule. This experiment lasted for 47 days and the animals performed 12000 +/- 4900 trials. One animal from this group reached a high performance on both trial types and therefore graduated into the stages with distractors. Two levels of distractor were tested: 19% and 25% for the visual distractor; and 18% and 31% for the auditory distractor. There was a drop of performance due to the distractor in both modalities, however the overall performance was still above chance levels and within an acceptable range. (Figures 11.B&C)

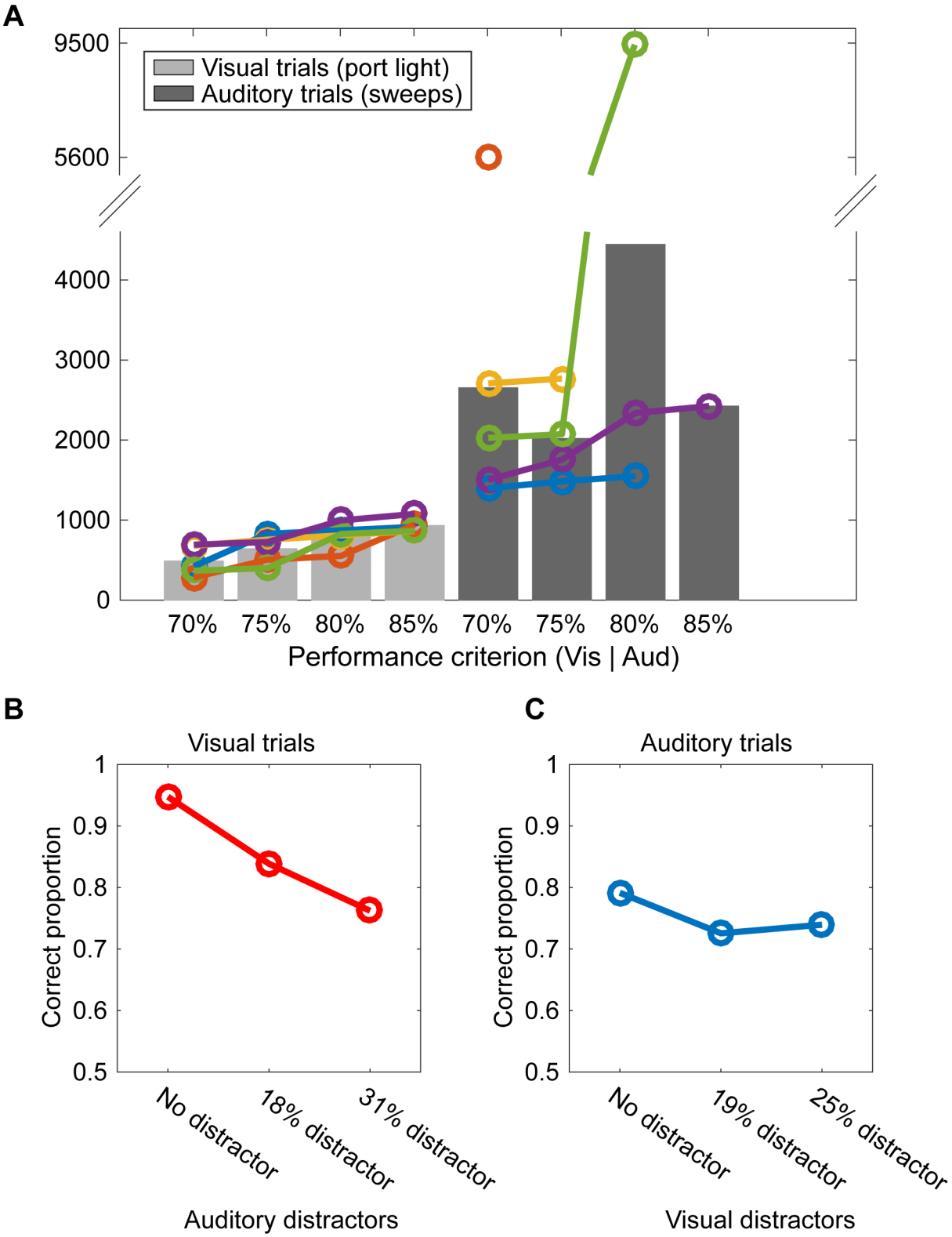

Figure 11. Animals can learn to respond to the stimuli in the presence of a distractor, and are capable of switching modalities. (A) The performance of animals in alternating blocks of visual stimulus (port light, light grey) and auditory (sweeps, dark grey) -- before the introduction of distractors. caption continued on next page →

7.6. Patterns of Day and Night Activity

Mice are known to be nocturnal animals. One of my findings during these experiments was that in the batches in which the animals had free access to the training chamber (no restriction on daily water), there was a pattern of activity correlated with day and night of the circadian system (Figure 12.A). Most of the activity occurred at night, just after the start of the dark cycle (6 pm). However the amount of activity was reduced in the last hours before morning (i.e. start of the day cycle at 6am). The minimum levels of activity were observed in the first hours of afternoon.

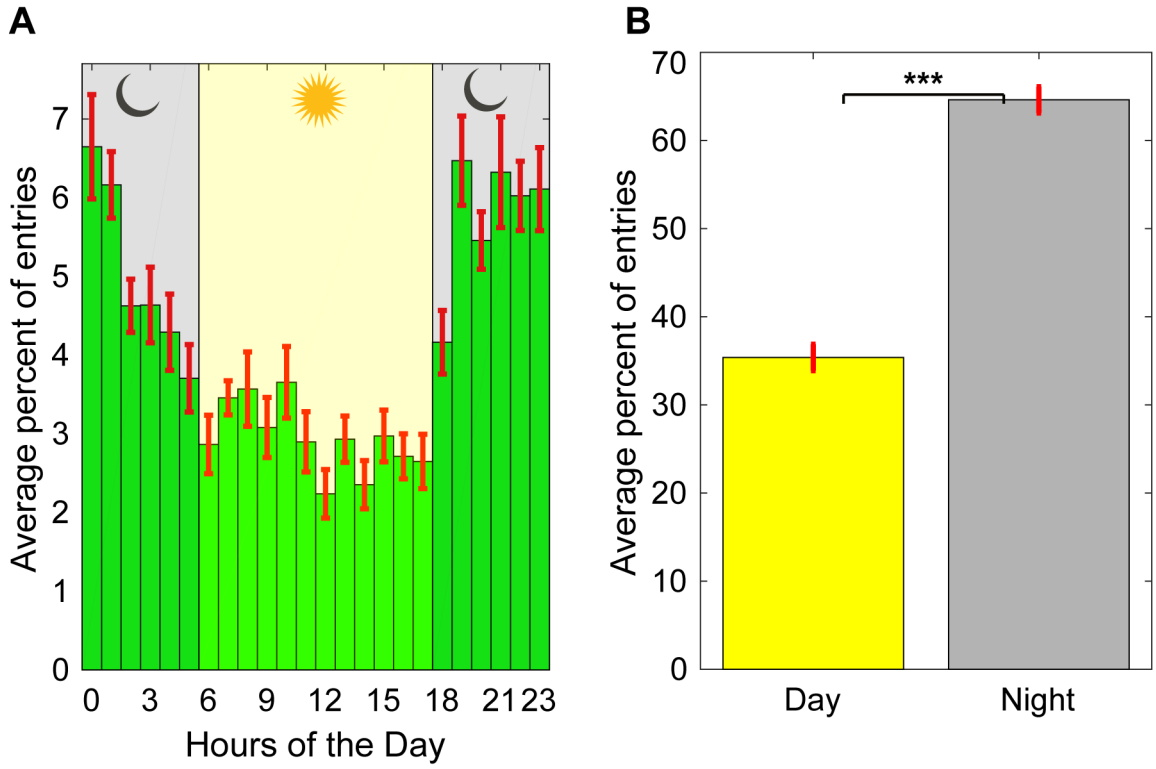

Figure 12. Mice are more active during the dark phase. (A) The average percentage of entries per hour of day. The daylight cycle is from 6 o'clock until 18 o'clock (shading). (B) Average percent of entries during day and during night (n = 22 in 4 batches, MWW test, p= 1.2 × 10-34).

→ (continued caption from previous page) (B) The performance of one animal in the presence of distractors. Auditory distractors were 52 and 57 db SPL sweeps. Visual distractors were 10 and 13 lx port light. main stimulus in the visual task was 52 lx and in the auditory task it was 67 dB SPL. Percentages for the auditory distractors are calculated based on sound pressure level formula; i.e. logarithmic scale.

8. Discussions

In this project I developed an automated training system for mice which is capable of individualized training of animals in a group housing, for tasks such as 2AFC, Go-Nogo, 5CSRT, etc. It has the capability of utilizing a diverse range of auditory and visual stimuli as well as reinforcers like air-puff and negative punishment (closing reward ports). I tested this system for training animals for the 2AFC task using light stimulus, pure tones, sweeps and gratings.

The methods developed and described in this thesis offer a promising path toward acquisition of complex tasks in mice. However the preliminary tests suggest that precise training strategies and step-by-step shaping of the behavior are necessary for a successful training.

8.1. Light as a conditioned reinforcer

I found that training the mice using the port light as a stimulus is fairly simple. However using this stimulus as a conditioned reinforcer to help them learn another stimulus was not successful. The main reason for this was likely that the light was being presented just at the end of the sound stimulus (2 sec). This was the time that in most of the trials the animals had already made a choice and responded to right/left. Therefore I do not know if I could have presented enough trials to associate the auditory stimulus with the light.

I also do not yet know if the concept of conditioned reinforcer works for mice or not; and if it works, whether it is possible to do it with light. However to test this approach we need a temporally more precise presentation of the light. Since my next approaches worked satisfactorily, I didn’t pursue this idea further. Nonetheless there might be other difficult tasks that need such an approach, but I don’t recommend it as a first hand solution.

8.2. Long stimulus time

Playing the stimulus for a long time after the initiation of the trial made it easier to train the animals for the 2AFC task. During the first trials, the animal has not yet associated the sound stimuli with an outcome, but likely rather adopt a simple trial-and-error strategy. The long stimulus presentation time may thus make it easier to form the association. The downside of this approach is that in the first stage the animals hear the sound stimulus many times without an association with a reward and this can reduce the contiguity between the stimulus and the reinforcer. However after the animal does a couple of hundred trials, the reaction time reduces to a time equivalent of 2 to 4 bouts of stimulus (0.5 s for each bout) which will eliminate this downside. Nonetheless the animal may learn to ignore the sound even in the first trials where it appears to be irrelevant to the task for them.

One approach might be tested to solve this. That is to first associate the sound with the reward for the animals in the introduction stage (just like I did for the light stimulus). So that the animal would receive reward by poking on any port, but before the reward is delivered one bout (i.e. one sweep) of stimulus associated to that port is presented. This can induce a contingency between the sound and the reward. With this approach when the animal graduates into the first stage of the 2AFC task, the sound already has a contingency and therefore can not be easily ignored by the animal.

8.3. Moving gratings

In this study I didn't succeed in training the animals for discrimination of visual gratings in a 2AFC task. I know that mice are not very visually guided animals (Baker, 2013), although there are studies which show that the mice can see and discriminate this type of stimulus (Burgess et al., 2017; Busse et al., 2011). The parameters in my study that might have led to this failure are the angle of gratings, monitor brightness/gamma adjustment and distance of monitor from the front wall. I used -110 and -70 angle degrees for the direction of the gratings' movements. These directions were adjusted to move toward the direction of the correct port. Other studies have used 0 and 90 angle degrees (Horizontal and Vertical) (Prusky and Douglas, 2004). It could be the case that the tilted gratings are not optimal for mice to perceive. Accordingly, in my further studies I will use the standard horizontal/vertical gratings. Mice can discriminate gratings from a longer distance than in my paradigm (for example in the tasks called "visual water task" in which the animal needs to swim toward the correct side of the maze based on the type of gratings presented at the end of the

corridor) (Prusky and Douglas, 2004). However the correct response in that case is located at the gratings location, so the animal needs to move toward it. This can associate the location with the gratings. But in my paradigm the gratings are further (~14 cm) from the location of the correct response and I speculate that this type of association might be more difficult. Based on this speculation, I propose that in further studies the monitor should be located just at the wall which the ports are located on.

In this study I didn't alter the brightness and gamma settings of the monitor. These monitors are originally produced for human vision, therefore I suspect that a high level of brightness can make it difficult for the animals to look at the stimulus or a low level of brightness might not be even visible to them. In this regard one should carefully calibrate the monitor for the mouse vision and try standard tests to make sure the mice can perceive the stimulus before going further on a more complex task. For example the optomotor response is a fairly good test of whether the animal can see the gratings or not.

8.4. The Requirement of Auditory Cortex for Auditory Stimulus Discrimination

Here I provide preliminary evidence that the auditory cortex might not be required for the recall of a previously learnt simple stimulus in the 2AFC task. This accords with previous findings in the lab using a fear conditioning paradigm. In order to validate this finding I need to increase the number of tested animals to see if the effect persists and if it is significant across animals. The next step would be to test the sweep stimuli to compare both groups to the fear conditioning paradigm and see if the effect is similar; i.e. higher decrease in performance for the sweep stimuli. To make sure that the effect is not due to other factors related to the optogenetics like the light of laser alone, I will also need a control group expressing GFP. The largest effect of laser (if it persists over enough animals) at 45 dbSPL, suggests that the auditory cortex is necessary for perception of even simple stimuli when the signal to noise ratio is low, potentially opening room for investigating the top down effects of attention.

8.5. Toward a Bimodal Attention Task

Here I managed to train one animal to discriminate both auditory (sweeps) and visual stimuli (light), in the 2AFC task amid the distractors from the other modality conflicting the stimulus. However to arrive at a completely bimodal task which can be used for assessing visual and auditory attention, I

need to go further. The final aim for such a task would be to incorporate moving gratings as the visual stimuli. I also need to increase the efficiency of the training to get more animals to reach the desired performance as well as making the training time shorter. In the final version of the task, the distractors should be increased up to 100% (of the learnt stimulus in the other modality) because the animal might learn to respond only to the more intense stimuli rather than learning the desired auditory/visual attention.

I have three proposals for improving the efficiency of this training. First and foremost, it is essentially important to switch to the non-dominant modality as soon as one task is mastered. My experience with training mice is that when they master a task, it is difficult to get them to do another behavior in the same context. Therefore one should not spend a lot of time on a task that showed adequate performance and should quickly switch to the second task. Second, adjusting the time of the cue which is needed for signaling the block/trial type (auditory or visual). Through some recent preliminary tests (data not presented here), I noticed that the cue might act as a stimulant for compulsive behavior. Mice often start to run for one of the two ports after the initiation of the trial (after hearing the cue) and not listen to what comes after; i.e. the stimulus. Also in the task introduced by Wimmer et al. (2015), the cue came before the initiation of the trial by the animal (i.e. holding the poke) just as a signal representing the upcoming modality of the trial, and not as a confirmation of the initiation of the trial like in my case. Therefore I propose that, in order to train the animals for such a task, the cue should be played at a time before the initiation of the stimulus. Third, incorporating punishment into the task: during this project I also developed two tools for punishment; an air puff as a positive punishment and a port blocker as a negative punishment. The air puff is aversive and has the downside of potentially discouraging the animals from performing the task at all. The port-blocker which consists of two lids controlled by a servo-motor and closes access to the ports, is designed to be safe for the animals but needs to be tested. Both are good candidates to test the effect of punishment in the learning process of the 2AFC task and potentially improving the efficiency of training.

8.6. Activity during the day and night cycle

I found that mice are more active during their night cycle and this accords with a previous finding (Remmelink et al., 2017). However one major confound to my finding is the human activity in the animal house. The animal caretakers start their work at 6am (when we see a drop of activity; i.e. start of day cycle). This confound can be addressed with changing the day and night cycle for the

animals by adjustment of light, and observe whether this difference persists. This finding suggests that the manual experiments should be adjusted to the hours with maximum activity of the animals rather than mere preference of the experimenter. This type of adjustment can potentially lead to higher yield of trials per animal.

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10. List of Abbreviations

2AFC: Two Alternative Forced Choice Task.

5-CSRTT: 5 Choice Serial Reaction Time Task.

ArchT: archaerhodopsin from Halorubrum strain TP009 (Han et al., 2011).

ATR: Automated Rodent Training System.

dB SPL: deci Bel Sound Pressure Level.

lx: lux (Lux): SI unit for measuring light.

PBS: Phosphate-buffered saline.

PFA: Paraformaldehyde.

RFID: Radio Frequency Identification.

TUNL: Trial-Unique Non-matching to Location.

11.Annexes

Disclaimer

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12. Acknowledgements

First and foremost, I want to thank Dr. Johannes Letzkus for his support of this project and providing valuable mentorship. I would especially like to thank Leona Enke for her peer support and her role as a main consultation point on animal behavior. I would also like to thank Verena Senn and Rogier Poorthuis for discussions and share of knowledge about training of mice, Tamas Dalmay for helping in preparing the optogenetics (including but not limited to surgeries and injections). Thanks you also Rogier for providing the ArchT data in slice, as well as everyone in the group for general discussions and share of knowledge and skills.

13. Declaration of authorship

I hereby confirm that I wrote this submitted master thesis myself without using any help besides the referenced sources and material. In every case where I have derived the words or meaning from other work, I have clearly indicated the source. The thesis or parts of it have not been published for either this or any other degree as examination material. I have received no remuneration for the scientific work performed in the context of this master thesis.

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