Behavioural mechanisms underlying rapid responses of rodents in an operant conditioning paradigm

A Thesis

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by

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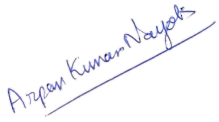
At the

Department of Biology
Indian Institute of Science Education and Research Pune

Certificate

This is to certify that this dissertation entitled "**Behavioural mechanisms underlying rapid responses of rodents in an operant conditioning paradigm**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Arpan Kumar Nayak at Indian Institute of Science Education and Research under the supervision of Dr. Nixon M. Abraham, Assistant Professor, Department of Biology, during the academic year 2022 January-December.



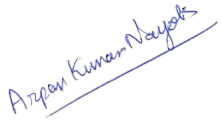
Dr. Nixon Abraham

Arpan Kumar Nayak

Declaration

I hereby declare that the matter embodied in the report entitled "**Behavioural mechanisms underlying rapid responses of rodents in an operant conditioning paradigm**" are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Educationand Research, Pune, under the supervision of Dr. Nixon M. Abraham and the same has not been submitted elsewhere for any other degree.

Dr. Nixon Abraham



Arpan Kumar Nayak

Contents

Contents

Abstract

In a natural setting, sensory awareness aids the animal in evaluating its surroundings and making appropriate judgments. The results of these choices have an impact on the animal's habitat survival and fitness. Rodents primarily rely on their olfactory system to get information and carry out necessary tasks for their survival, including finding possible mates, foraging, navigating, seeing predators, etc. Based on a well-known Go/No-Go olfactory behavioural paradigm, we train mice in our lab to execute detection and discriminating tasks. Water-deprived animals learn to lick for rewarded stimuli (reward being water) and to refrain from licking for non-rewarded input. Animals gradually develop the ability to distinguish between odour stimuli that are rewarded and those that are not. However, a preliminary study from the lab shows that animals that execute as accurately as possible respond to certain unrewarding stimuli by licking and quickly stop responding. These quick licking reactions may be the result of inadequate stimulus percept generation, in which a preliminary judgement was made prior to thorough processing and integration of the incoming stimulus. In order to characterize this behavior, we carried out Go/No-Go odor discrimination tasks and established a means to record these anomalies as reversal trials with its own set of characteristics. Further experiments were carried out to look at the significance of an odor vs a diluent in an odor discrimination task. This project aimed at attempting to quantify and characterize the properties of reversal trials and this phenomenon overall. Our findings call for further experiments to dissect out the physiological mechanism and behavioural impact of the same which will help us establishing this property as a usable readout to quantify finer subtleties in the decision making process.

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1. INTRODUCTION

In their natural habitat, sensory perception helps organisms to comprehend the external world and make decisions accordingly (Churchland et al., 2008; Hanks & Summerfield, 2017). The results of these regular decisions affect fitness and survival. The events in question could be as difficult as figuring out the best way to flee from a predator or as perceptive and complex as choosing the right moment for an interhemispheric migration, as the arctic tern's annual journey from pole to pole (Alerstam et al., 2019). Sense of smell is the primary sensory organ in rodents. They depend on their sense of smell to gather information and carry out survival-critical tasks including navigation, foraging, spotting possible mates, sensing predators, etc (Wilson, 2008; Wilson & Mainen, 2006).

1.1. PERCEPTUAL DECISION MAKING

The process of decision-making involves assessing different physical, emotional, and social parameters that could influence choice selection. Apt behavioral responses emanate from the formation of a stimulus percept in the decision-making process which helps the organism to be cognizant of its immediate surroundings. Another factor that comes into play while dealing with sensory information from real-life situations is its noisy and dynamic nature. Over this are the factors of internal and external states that might affect the course of action taken. Environmental biases and the factor of the flexibility of the cognitive processes add another layer of complexity to that. A coherent functioning between the sensory system and the internal reinforcement has been shown to accelerate learning and hence improves decision-making abilities (Milman et al., 2019). For sensory stimuli such as that of vision, it is well established that the decision-making process is influenced by the temporal integration of sensory inputs since it has a role in the formation of stimulus percept formation (Dick et al., 2001; Moher & Song, 2014). However, we can’t say the same for a sensory modality that is chemically derived. For example, the ability of animals to identify and classify olfactory stimuli in their natural habitat is equally important to make accurate decisions. Hence the information on how odor signals are perceived and processed in the olfactory bulb and also in the higher cortical areas is necessary to comment on the dynamics of the decision-making process in the olfactory context.

1.2. RODENT OLFACTORY SYSTEM

Olfactory information processing begins in the epithelium of the nasal cavity with the odorant molecules binding to their respective olfactory receptors (ORs)(Buck et al., n.d.; Schaefer & Margrie, 2007) on the surface of the olfactory sensory neuronal cilia (OSNs), each expressing only one olfactory receptor, out of the 1300 ORs known for mouse olfactory system, except for a very few OSN which can express more than one receptor type (Buck et al., n.d., Tan et al., 2015). The epithelium also includes the sustentacular and the basal cells (Graziadei & Graziadei, 1979; Xie et al., 2013). The binding of the odorant molecule with the receptor sends out information along the OSN projections to the Olfactory bulb (OB).

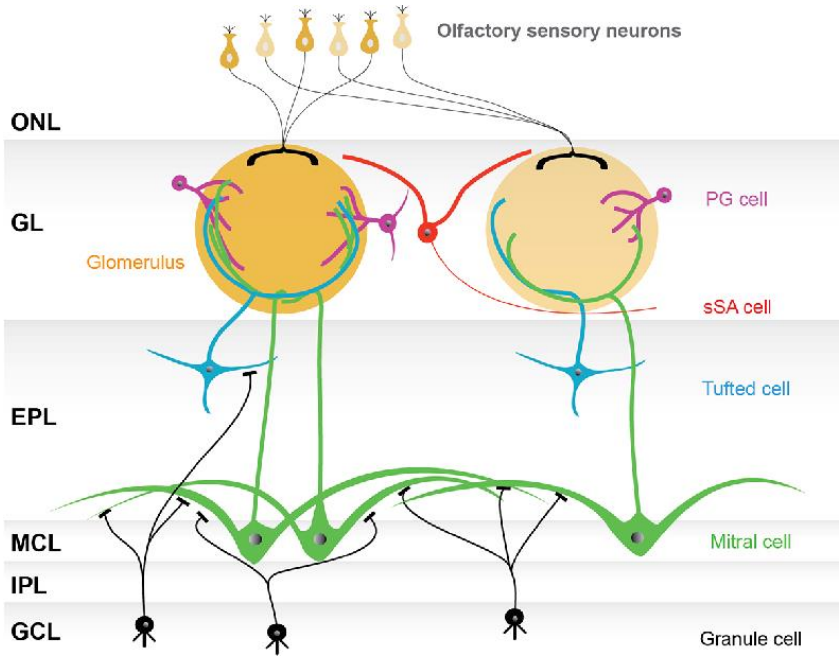


Figure 1: Schematic diagram of the layered structure of the olfactory bulb (Nagayama et al., 2014)

OB, a part of the forebrain is a laminar structure (Figure 1) with a diverse cell population, starting from the outside to the innermost layer: olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL) and the granule cell layer (GCL) (Macrides & Schneider, 1982; Mori et al., 2009; Nagayama et al., 2014; Shepherd et al., 2007) Projections

from the OSNs form spherical neuropil-like structures called glomeruli (in the GL). Notably, for any given glomerulus, all the OSNs that terminate into that particular glomerulus, express the same ORs (Mombaerts et al., 1996; Mori & Sakano, 2011; Soucy et al., 2009). These ORs can recognize multiple odorant molecules and vice versa i.e. multiple odorant molecules can be recognized by a single OR. Hence, different odorants are perceived by a combination of ORs (Malnic et al., 2000).

1.3. STIMULUS PROCESSING IN THE OLFACTORY BULB

OB is the site of primary olfactory sensory information processing. Information from the bulb is transmitted to the olfactory cortex by projection neurons from the MCL and the EPL. Their primary dendrites synapse in the glomeruli with the OSNs. However, before reaching the higher brain centers, odor information is processed within the bulb (Gallarda & Lledo, 2012; Wilson, 2008). This involves signal transduction in the GL, with the help of the juxtaglomerular cells (JGCs), chiefly interneurons such as superficial short-axon cells (sSACs), periglomerular cells (PGCs) and a subpopulation of external tufted cells (ETCs). Hence, different cell types of the OB communicate with each other across different layers with the help of projection neurons forming dendrodendritic synapses. The sSACs and the PGCs decrease the activity of the projection neurons owing to their inhibitory nature (Burton, 2017; Burton & Urban, 2014). To have a precise percept formation and an efficient decorrelation of overlapping patterns of sensory stimuli in the glomerular layer, a proper balance needs to exist between the excitatory and inhibitory activity in the OB (Barnes et al., 2008; Gschwend et al., 2015). Also, the factor of combinatorial coding of odor information in the OB makes the olfactory system one of a kind (Malnic et al., 2000). Additionally, for the olfactory context, it has been shown recently that the decision-making process is influenced by the complexity of the input stimuli (Bhattacharjee et al., 2019).

1.4. DECISION REVERSAL IN GO/NO-GO TASK

The Go/No-Go task is a simple behavioral paradigm that requires the participant to do exactly what it says: respond to the “Go” signal and not respond to the “No-Go” signal (Abraham et al., 2004). The nature of the input signal and response elicited varies depending on the context of the experiment and the modality involved. This is a very

handy tool to study the decision mechanism and in the olfactory context, multiple variants of this concept have been used (Loos et al., 2010). For example, it has been seen when a Go cue is followed by a Stop signal, with a varied delay interval in between both cues, behavioral outputs tend to terminate and this phenomenon is called the stop-signal task (Mayse et al., 2014, 2015). A similar yet peculiar behavior is observed in the Go/No-Go paradigm used in our lab: the go cue and no-go cue are provided to the participant (here mouse head-restrained to the setup) in a pseudo-randomized manner. It has been repeatedly observed that there is a distinct deliberate cessation of the motor response for the non-rewarded stimulus (the no-go cue). And this stopping response always occurs after the initiation of the response. The task demands water restrained animals to respond to the go-cue by licking onto a water delivery port placed near their mouth; also the site from where they receive water as the reward. When animals are trained on a Go/No-go behavioral paradigm for an odor discrimination task, they begin with licking in response to both the rewarded and the non-rewarded stimulus (go as well as no-go cue). Over the training sessions, they start associating reward availability with stimulus i.e. for rewarded odors and refrain to lick for non-rewarded odors. As training progresses, the performance accuracy reaches upto 80%. Intriguing observation is that the learnt animals that are performing with higher accuracy exhibits a tendency to start licking for the unrewarded trial initially followed by stopping of response after few ms of odor presentation. These lick responses often begin with stimulus commencement, or a few milliseconds after odor delivery, and are then suppressed later in the stimulus duration. These non-rewarded trials are considered as Failed to No-Go trials. These trials also constituted error trials in which animals held on the tube for longer due to motivation issue or technical glitches. Thus, after excluding error trials all failed to No-Go trials were considered for analysis of lick behaviour and called as Reversal/Revision trials.

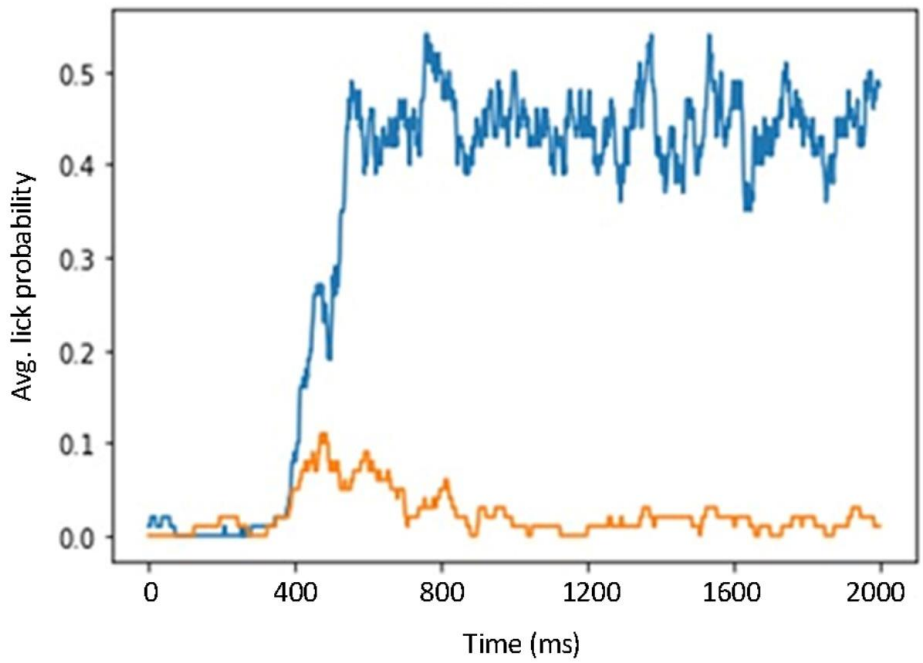


Figure 2: Lick probability plot for a single animal (blue- S+ trial and orange- S- trial)

Figure 2 shows the average lick probability of a single animal having already learnt the paradigm hence performing the task with an accuracy well above 80%. The plot shows the lick patterns for both the rewarded as well as the non-rewarded stimuli. It can be noted that there is a distinct peak on the non-rewarded stimulus at the point of deflection of both the curves. This feature was noticed for almost all animals across different experimental settings. This represents a behaviour at the population level for the no-go cue by animals that have already learnt the task. We hypothesised that these short licking behaviour as a response to the unrewarded stimuli might be emanating from incomplete stimulus percept formation i.e. a primitive decision is made before the complete processing and integration of the incoming stimulus, which later gets revoked upon a full percept formation, in the later phases of stimulus duration.

In the current work, we aimed at characterising this peculiar behaviour of the non-rewarded trials and establish these responses as a quantitative behavioural readout. We also looked at the dynamics of this behaviour in the context of altering stimulus percept formation in an olfactory detection and discrimination task. To start with, we quantified these and developed a robust method to extract such revision trials with high temporal resolution. We establish further sub-parameters to look at the factors which can be used to characterise the properties of these trials under different

experimental conditions involving wild type animals (C57BL6/J) and mouse model with perceptual deficits (mouse model of stress). Further we adopted different experimental strategies to look at how these properties change based on the presence or absence of odor as a stimulus and how complexity of the task plays a role in framing the features of these decision-reversal trials.

2. MATERIALS & METHODS

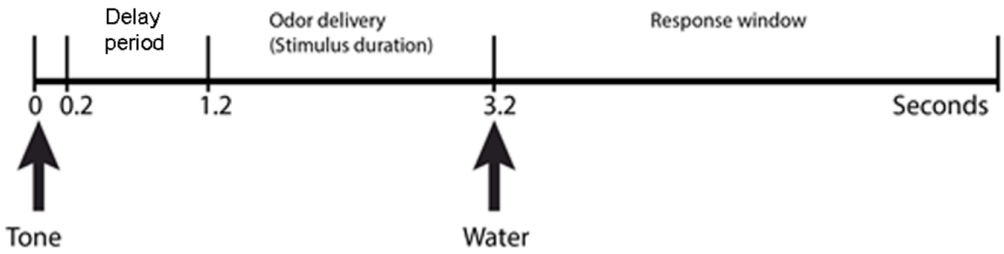
2.1. ANIMALS USED

Mice of the C57BL/6J (Jackson Lab) wild type, ranging in age from 8 to 10 weeks, were used in all behavioural studies. Metal head-posts were implanted using a stereotaxic system, followed by a two-day recovery period before behavioural experiments. Animals were housed in individually ventilated cages (IVCs) on a 12 hours light-dark cycle with 50-60% relative humidity and 25-27 C temperature, and ad libitum food and water while the experiments were not being conducted. Animals were water restricted for 12–14 hours a day, five–six days a week, during behavioural experiments. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Ethics Committee, IISER Pune, and the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

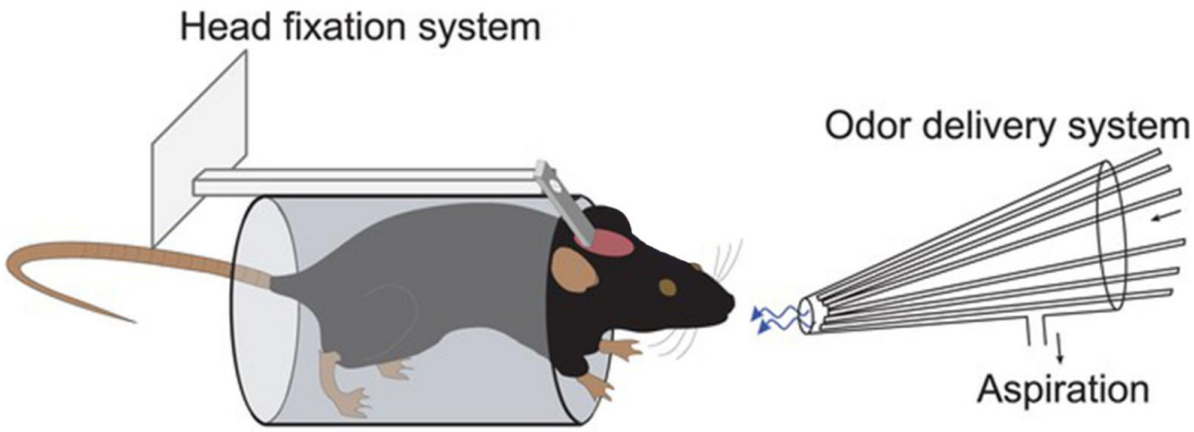
A total of 20 wild-type mice were used for the experiment, 10 in each group of experiments. Results does not include animals that couldn't finish all the tasks or with failed head post implantations.

2.2. GO/NO GO BEHAVIOR PARADIGM

The behaviour experiments were performed by training mice on custom-built Olfactometers, using the Go/No-go operant conditioning behaviour paradigm. Training was preceded by a pre-training phase which helps the animal to get acquainted with the setup. Pre-training helps the animal to get accustomed to the setup gradually learn lick criteria to correct hit trials (rewarded trials). Initial phases of pre-training are designed to make animals lick on lick port for obtaining water as reward, followed by subsequent phases wherein air puff/neutral stimulus (mineral oil) is introduced. Animals are required to lick during stimulus presentation time to secure 3-5µL of water per trial as reward. Figure 3 shows an illustration of the trial structure. Pre-training phases constitute only rewarded trials. This criterion is made progressively stringent so as to match the actual training criteria, over successive trials.



(a)



(b)

Figure 3: (a)A representative diagram of a standard trial. It starts with a short tone of 200ms followed by a delay time of 1 second. Odor is then delivered for a 2 second duration which is followed by the response window. At the end of odor-stimulus duration, water is delivered as the reward for a rewarded trial (S+ trial); (b) Schematic illustration of the head-restrained setup for behavioural experiments (Abraham et al., 2014)

During the training phase, the animal gets reward only for one out of the two odour saturated air puffs. The air puffs were provided by an aquarium pump at a steady flow rate of 400mL/min. The training/conditioning phase involves the mouse to discriminate between the rewarded (S+) and the non-rewarded (S-) stimuli and respond accordingly to obtain the reward. The animal is placed inside of a cylindrical tunnel, at the mouth of which the animal's head is fixed using a screw that attaches the head post on the animal to the anterior part of the tunnel. The nozzle of the odour port is

placed right under the left nostril of the mouse at an approximate distance of 3mm. The lick port is placed under the mouth of the animal at roughly the same distance. The base of the tunnel has a wire mesh which acts as a conductive interface. Every time, the animal licks the metal tip of the lick port, it acts as a key completing an electric circuit, which feeds the information back to the computer at a high temporal resolution. Every trial begins with a brief 1s long preloading time followed by the onset of odour delivery which lasts for a total of 2s, and also serves as the response window at the end of which water is rewarded if the conditions are met. Rewarded (S+) and non-rewarded (S-) trials, 10 of each sort, are distributed in blocks of 20 trials in a pseudo-randomized way. This is done so that not more than two consecutive trials of S+ or S- are presented together in each block. The stimulus duration of 2 seconds is divided into 4 bins of 500ms each. For the S+ trial to be deemed as correct, animals have to lick at least once, in minimum 3 out of the 4 bins (or a minimum of 240ms as the lick duration). For S- trial to be correct, animal has to refrain from licking or it must not lick for more than 1 out of the 4 bins (or a maximum of 80ms of lick duration). There is no negative reinforcement or punishment of any kind involved for the wrong trials. There is an 8-9 seconds long window during which the animal obtains the reward (for correct S+ trials), before which the next trial is initiated. Additionally, if the animal licks during the preloading time (i.e. before the odour delivery), double the duration of lick is required during stimulus presentation to obtain reward in a S+ trial.

2.3. HEAD POST IMPLANTATION

The GO/No Go behaviour experiments were performed on the head-fixed setup, where the animals were restrained using a stainless steel head post. The head post is implanted on to the cranium of the animals. Animals were intraperitoneally administered a mixture of Ketamine and Xylazine (in the ratio of 36:15) as anaesthesia; the dosage being equated according to their body weights as 2µL per gram of body weight. Surgical procedures were performed once animals were deeply anaesthetized and unconscious i.e., absence of reflex upon toe pinching/ no whisker movement. A small part of their tongue was retracted out of their mouth, so that there is no hazards of choking. Animals were placed on the stereotaxic setup (with labelled ear & nose bar) to avoid head movement. Clinically prescribed eye drops were used to keep them hydrated and prevent corneal drying during the head post implantation.

A solution of artificial cerebro-spinal fluid (ACSF) was used during the cleaning and the moistening of the exposed tissues.

Lidocaine was applied on the head of the animal, below the eye level and sterilised surgical tools were used to make a small circular opening in the skin on the head about 1cm in diameter. The exposed transparent sheath of connective tissues (Periostuem) was cleared off. A few drops of etching cream (Ivoclar Vivadant EcoEtch) was put on the dry cranium to partially corrode the cranium surface creating a scratchy surface. Next a dental primer (Ivolclar Vivadant Te-Econom Bond) was applied which acts as the intermediate agent. UV treatment was used to dry it. A thin layer of white dental cement (Ivoclar Vivodant Tetric N-Ceram) was spread and a surface equipped with a grip was created for the head post to have friction while being placed on it. It was UV treated from a close proximity so that cross-linking instantly hardens it. Another thin film of white cement was applied at the base and sides of the stainless steel head post and was fused to the already hardened cement layer at the base, following by adequate UV treatment. Dental acrylic cement (DPI RR Cold cure) was then used to seal all the exposed parts of the cranium fusing and hardening with the skin in a couple of minutes, marking the cessation of the head post implantation. The animal was then monitored till the effect of anesthesia was gone.

2.4. Data Acquisition and Filtering

The data produced by the behavioural experiments are acquired and stored in separate csv files with the help of custom made C++ files. Every trial is roughly 13 seconds long; blocks of 20 trials (that have 10 of each kind: S+ and S-) are created and the percentage accuracy is calculated block wise to determine how efficiently the animal has learnt to discriminate between the rewarded and the non-rewarded odour in the experimental task.

This data includes 3 kinds of files:

Block-data: It stores data about the time taken for every block, the block number, number of trials performed in every block, the session number, the number of trials that were performed correctly vs incorrectly for S+ as well as S- stimuli per block, the accuracy of the performance in percentage and the total block-wise accuracy attained

(averaged over both the S+ and S- trials). This data is segregated for every session that the animal was trained.

Begin Time	End Time	Session nb	Protocol nb	Trials	S+ OK	S+ NOK	S- OK	S- NOK	% S+	% S-	% Total
05-17-2017 11:44:22	05-17-2017 11:49:30	1559	1	20	5	5	9	1	50	90	70
05-17-2017 11:49:30	05-17-2017 11:51:03	1559	2	6	0	2	4	0	0	100	66.67

Figure 4. A snapshot from the data acquired and recorded in the block results file. The red box represents an example of a complete block

Trial-data: It stores data about the timing of every individual trial, the trial number, the type of trial (rewarded or non-rewarded), whether the particular trial was performed correctly or incorrectly and whether reward was delivered at the end of the odour presentation. The odour is given for a total of 2 seconds every trial. This period of 2000ms is divided into 1000 equal bins and the state of circuit being complete or not i.e. if the animal was licking or not, is recorded for every bin as the 'lick state' (1 for a lick and 0 for a no-lick). This data is also segregated session wise.

Time	Session nb	Protocol nb	Trials	Type	LED State	Result	Rewarded	bin 1	bin 2	bin 3	bin 4	bin 5
05-17-2017 11:44:37	1559	1	1	S+	0	PASS	1	0	0	0	0	0
05-17-2017 11:44:52	1559	1	2	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:45:08	1559	1	3	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:45:23	1559	1	4	S+	0	FAIL	0	0	0	0	0	0
05-17-2017 11:45:39	1559	1	5	S+	0	PASS	1	0	0	0	0	0
05-17-2017 11:45:54	1559	1	6	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:46:09	1559	1	7	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:46:25	1559	1	8	S+	0	FAIL	0	0	0	0	0	0
05-17-2017 11:46:40	1559	1	9	S+	0	PASS	1	0	0	0	0	0
05-17-2017 11:46:56	1559	1	10	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:47:11	1559	1	11	S+	0	PASS	1	0	0	0	0	0
05-17-2017 11:47:27	1559	1	12	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:47:42	1559	1	13	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:47:57	1559	1	14	S+	0	FAIL	0	0	0	0	0	0
05-17-2017 11:48:13	1559	1	15	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:48:28	1559	1	16	S+	0	FAIL	1	0	0	0	0	0
05-17-2017 11:48:44	1559	1	17	S-	0	FAIL	0	0	0	0	0	0
05-17-2017 11:48:59	1559	1	18	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:49:15	1559	1	19	S+	0	PASS	1	0	0	0	0	0
05-17-2017 11:49:30	1559	1	20	S+	0	FAIL	0	0	0	0	0	0
05-17-2017 11:49:46	1559	2	1	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:50:01	1559	2	2	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:50:17	1559	2	3	S+	0	FAIL	0	0	0	0	0	0
05-17-2017 11:50:32	1559	2	4	S+	0	FAIL	0	0	0	0	0	0
05-17-2017 11:50:48	1559	2	5	S-	0	PASS	0	0	0	0	0	0
05-17-2017 11:51:03	1559	2	6	S-	0	PASS	0	0	0	0	0	0

Figure 5. A snapshot from data acquired and recorded in the form of trial data. The red box represents an example of a complete block corresponding to the same block from figure 4

Master-file: This is the largest file that stores data in an extensive manner. It records every minute alteration, such as changes in lick detection state, the licking duration, the breath count, liquid and odour, the bin number, etc. time stamped with a temporal precision as high as 30 microseconds.

Custom Python programming scripts were created in order to extract the pertinent trials from these data sets. Only trials that were a part of complete blocks were taken into account for analysis (block with 20 trials). These blocks were again filtered based on average percentage accuracy and blocs with 80% or above accuracy were considered. In few trials, animals tend to hold on to the lick port or parts of their snout come in contact with the lick port. These situations could give some false signals for the lick detection states. In order to avoid taking in any such kind of trials for the analysis of potential decision reversals, a criterion for maximum single-lick duration was set. For every trial, each lick's duration was taken into account and all such trials were excluded in which a single lick was longer than 80ms in duration. This criterion of 80ms was determined based on literature references and iterative calculations in which all individual lick durations were averaged over all rewarded and non-rewarded trials separately to calculate a representative value for a single lick duration (mean = 42ms, SD= 12).

Further, a representative distribution of licks in the stimulus window of 2 seconds, animal-wise, was produced by cumulating the lick states for all the bins (2ms each) of the filtered trials across all the animals in the set and normalising them. The normalisation step is done in order to avoid the representative lick plot to be a numerical representation of the number of reversal trials (RTs) per animal. The average of the lick states was then calculated for all the animals.

2.5. BEHAVIOURAL READOUTS

Learning curve: Animals were trained on an odor discrimination task to differentiate

between 2 odors. They had to perform trials that were presented in groups of 20 called blocks that had an equal number of S+ and S- trials and the average accuracy was calculated block-wise. Learning curve gives the progressive performance of animals across trials. (Fig 6a)

Lick pattern: Animals tend to lick for the S+ and refrain from licking for the S- trial respectively and this gets refined over time. When we take the average of about 100-200 trials of S+ and S- separately and plot them across the odor duration time, we get the representative probability of the lick states bin wise (1 bin= 2ms, total of 1000 values for 2s) (Fig 6b)

Detection and Discrimination time: When we take the bin wise values of a large number of trials, and then compare the lick states of the S+ trials against those of the first few milliseconds (25bins/50ms), we can do statistical tests to find out the point in time when the lick probability deflected significantly for the first time from the baseline value. That gives the detection time of the animal. When we carry out similar tests between the S+ and the S- trials (having considered an equal number of each), the point in time where they significantly divert from each other that point is known as the discrimination time. Figure 6c shows the p-curves obtained from t-tests performed on the lick states across bins.

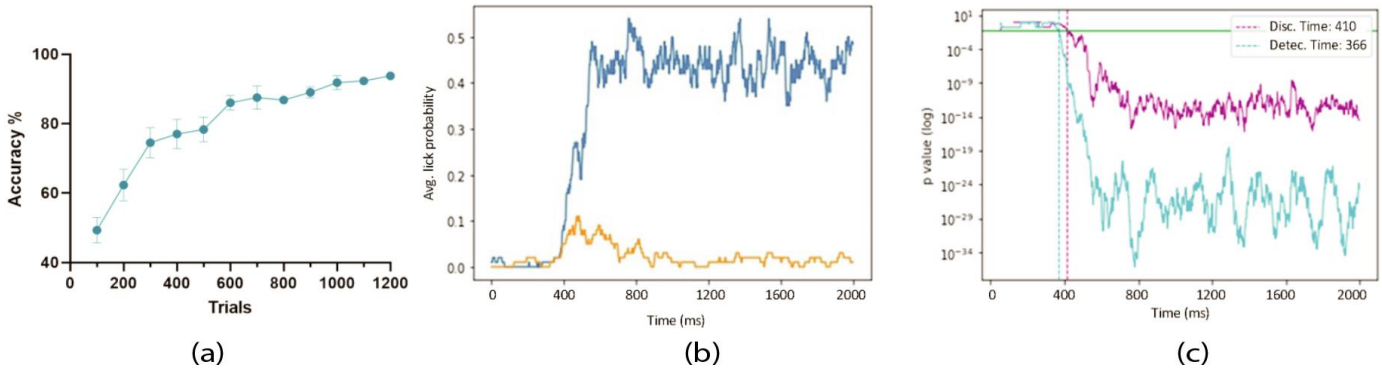


Figure 6: (a)Learning Curve for monomolecular odor discrimination task average of 7 animals, whiskers depict SEM; (b) Lick pattern average: average of lick probability states of 1 animal with accuracy >=80%, (c) p-curve from one-tailed t-test for the same set of animals

2.6. DATA ANALYSES

The first 50ms of lick state was taken as baseline licking and a bin wise one-tailed unpaired Student's t-test was performed between baseline licking and every successive bin to determine the boundaries of the peak, if any. This was all performed in python using available packages: Numpy, Matlpotlib, Pandas and Scipy. The fraction of reversal trials was calculated out of the total number of S- trials that belong to blocks with at the least 80% average accuracy. This window obtained from the p-curve is used to see how many trials from among the reversal trials existed such that their individual licks primarily lied within this window and this attribute was called reversal trials in the window (RTWs). Analysis for the reversal trials in the freely moving setup is done similarly keeping all the parameters just the same.

Graphpad Prism software was used to perform all statistical analyses. This also includes the plotting of figures showing bar graphs, pie charts and smoothed average lick states for reversal trials. For comparison of curves obtained from averaged lick states of reversal trials, KS (Kolmogorov-Smirnov) test was used. K-50 Nearest neighbour smoothing was applied to the curves just for a better visual representation, while the actual t-test and KS test was done on the raw data. This smoothing works by averaging/differentiating data points in the neighbourhood of the said size. K=50 was found to be a desirable size since that represents only 5% of the total number of data points (50 out of 1000) and is still large enough to yield a smoother curve. Analysis of the breathing data was primarily done using custom made Python scripts. The breath sensors record data with a distinctly high temporal resolution based on a thermocouple mechanism. This data is saved as binary information which can be used to determine inhalation onset, sniff frequency, etc. This information was compiled over trials in Python and then statistically analysed using Graph Pad prism.

3. RESULTS

3.1. PRELIMINARY RESULTS

It has been shown that rodents learn to distinguish between odor stimuli based on reward association in an olfactory Go/No-go paradigm within first 150-200 trials. The discrimination time is the time during odour presentation when the lick probabilities of the rewarded and unrewarded stimuli markedly diverge (Figure 7). The lick pattern of the non-rewarded stimulus spikes up a bit, around the discrimination time within the odor duration (total duration of 2 seconds) and fades away after a few milliseconds into odor presentation. It has been observed repeatedly across experimental designs that there exists a definitive peculiarity in the average lick pattern of the animals for the non-rewarded trials. As mentioned before, this peculiar behaviour persisted across different odor discrimination tasks in an olfactory Go/No-go setting.

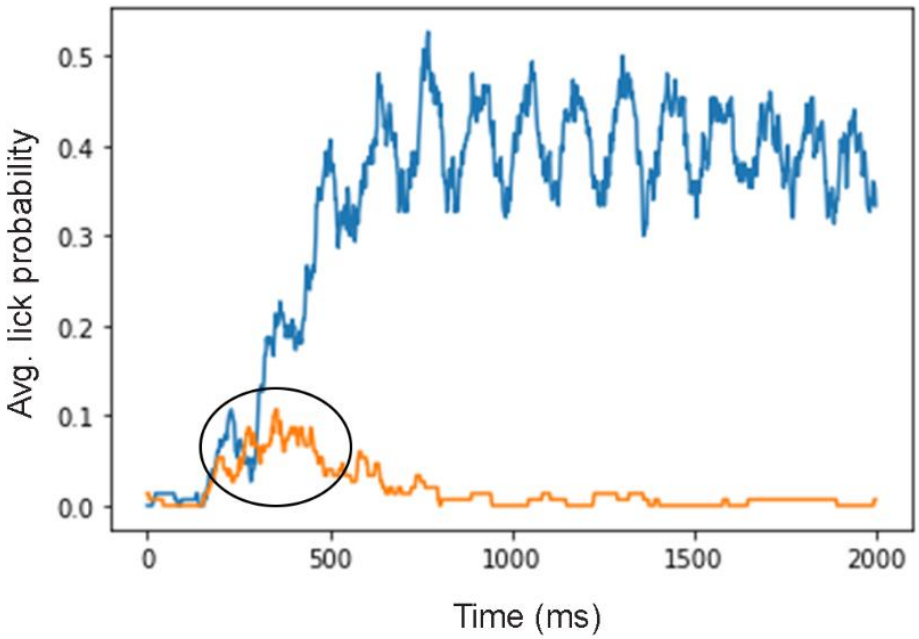
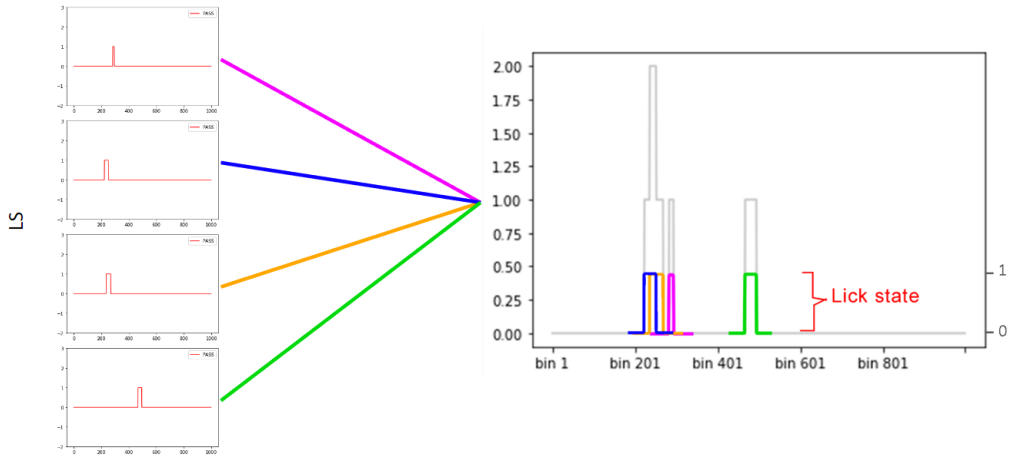
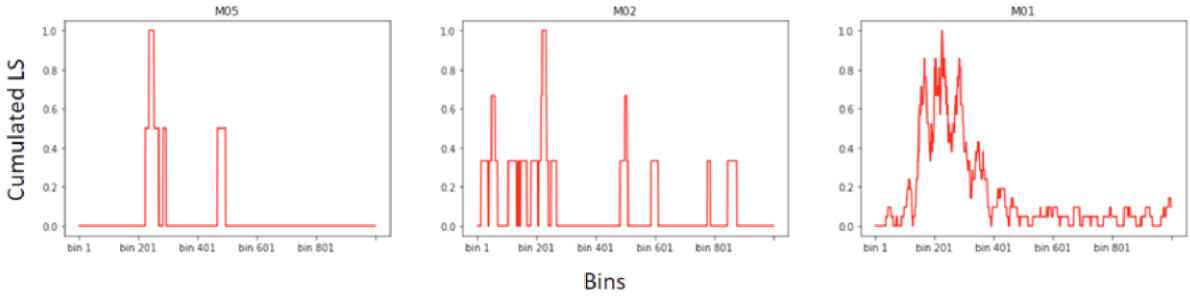


Figure 7: Avg. lick probability states showing the peculiar bulge on the lick pattern for the non-rewarded trial (orange)

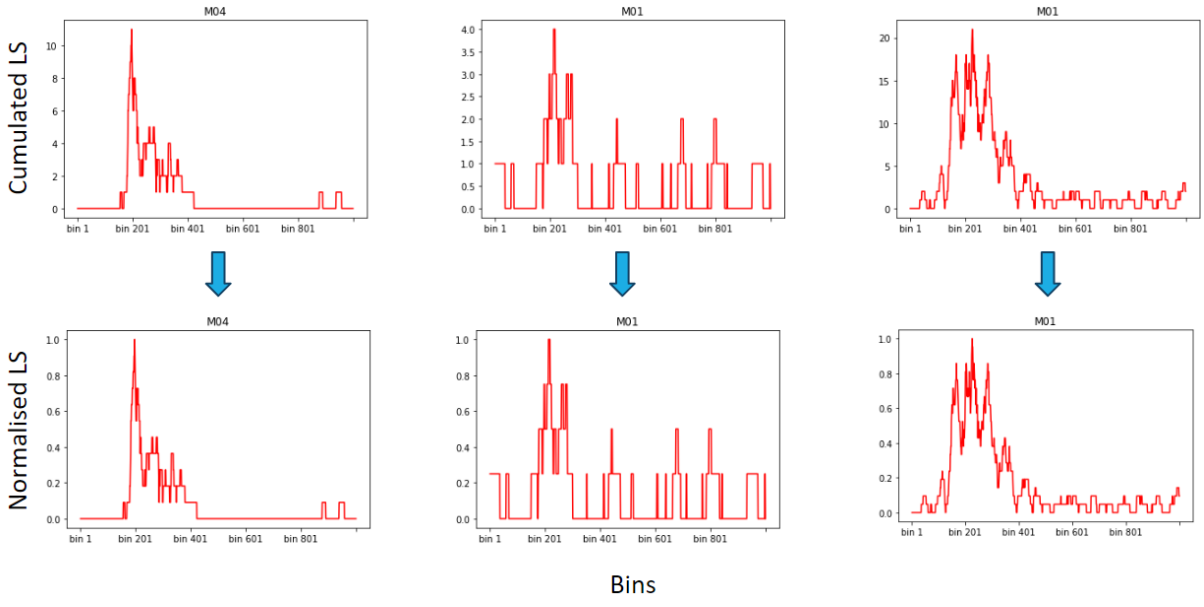
In order to quantify this licking behaviour of mice for the (non-rewarded) S- trial, we decided upon a few filtering criteria to take only the relevant trials for the analysis, henceforth called reversal trials (RTs). In order to get a gross representation of just the RTs, we accumulated them bin-wise to obtain the cumulative lick plot of every animal as shown in Fig. 8.



(a)



(b)

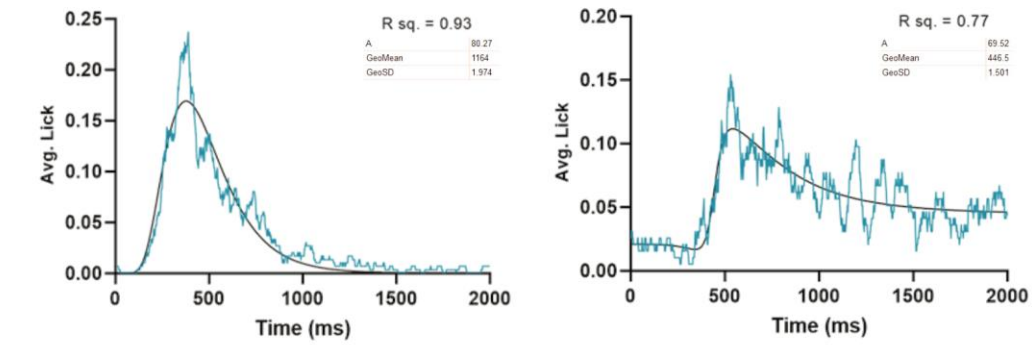


(c)

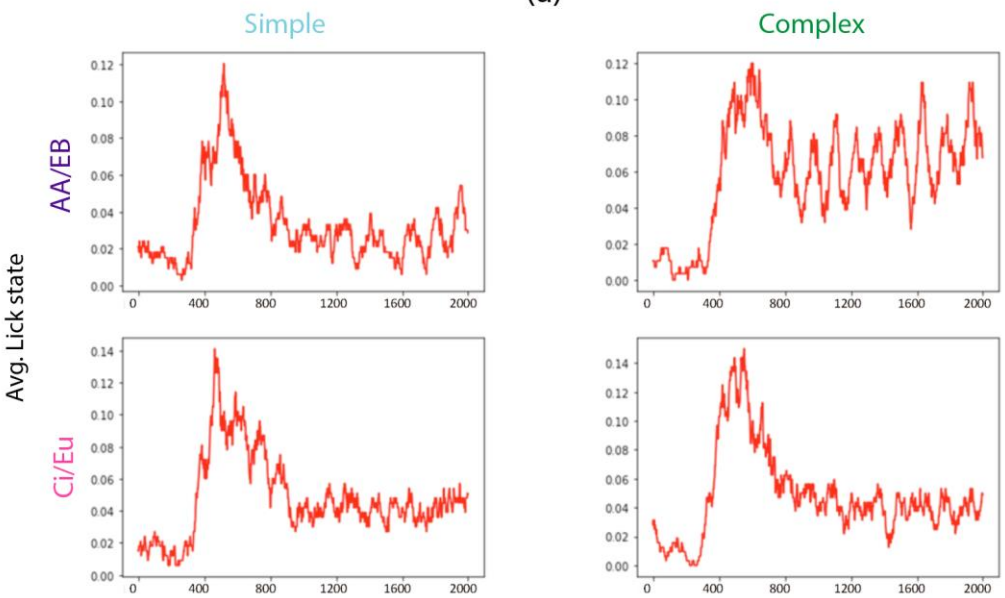
Figure 8: Steps involved in data processing and curve generation;(a) shows the cumulation step with bin wise simple addition of lick states for 1 animal; (b) relation between the number of RTs and the curve characteristics (4RTs vs 16RTs vs 64 RTs) for 3 different animals after execution of all filtering criteria;

(c) Normalisation step done by dividing all values by the maxima animal wise

It can be seen that the features of the plot for example smoothness and peak value of the cumulated plots is dependent on the number of the RTs, hence before averaging these plots for all animals in the same experimental group, we normalized them based on the total number of these reversal trials, in order to remove their influence on the characteristic of the curve generated. We roughly got 5-20% of such trials over different experiments performed. This could lie in the range of about 20-60 RTs in general per animal totaling up to about 200 trials in an experimental set. Following this they were averaged, to yield a representative plot for every experimental group. It was observed that there is a distinctive peak in the first half of the stimulus window. This data could fit well with a log-normal distribution (Fig. 9a).



(a)



(b)

*Figure 9: (a) Average of reversal trials fitted with a lognormal distribution on 2 different data sets- black line represents fitted curve, green line represents raw data, eqn.: Y=(A/X)*exp(-0.5*(ln(X/GeoMean)/ln(GeoSD))^2); (b) Compilation of data showing similar patterns in RTs from experiments done previously in lab*

We performed similar data extraction and analysis with data produced in lab in previous experiments and found the peak with similar curve characteristics (Fig. 9b). Hence, we came to the conclusion, that this peculiar behaviour that only accompanies the non-rewarded stimuli is a recurring behaviour that exists in Go/No-go olfactory discrimination tasks across different experimental conditions.

3.2. Quantification of Reversal trials

In order to establish this parameter as a working readout, we decided to alter various attributes of this behavioural phenomena (mostly the number and time duration) by making relevant changes in the paradigm. Hence, two sets of naive animals were taken and odor discrimination tasks were performed in the sequence as shown in Figure 10.

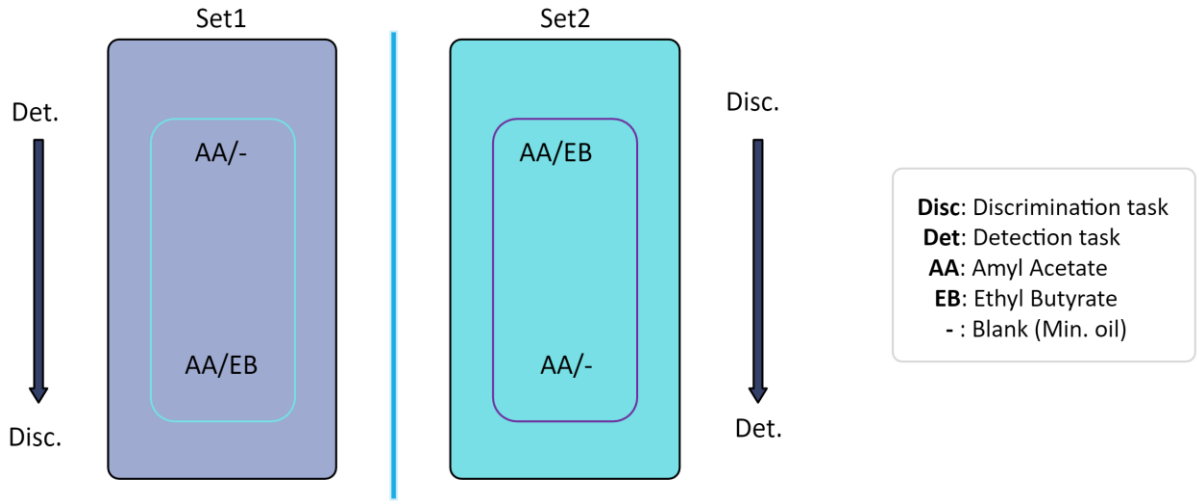


Figure 10: Schematic diagram of experimental paradigm showing the different group of animals (Grp1 and Grp2) included and the chronology of experiments carried out

Animals were trained for 900-1200 trials over a span of 10-12 days. They reached performance criterion in first 150-200 trials. By performance criterion it is referred to the average accuracy of the animals' performance in a given block of 20 trials. The average of their learning curves were plotted to confirm the same. Similar criteria were

used to filter the data and trials were acquired to determine the reversal trials.

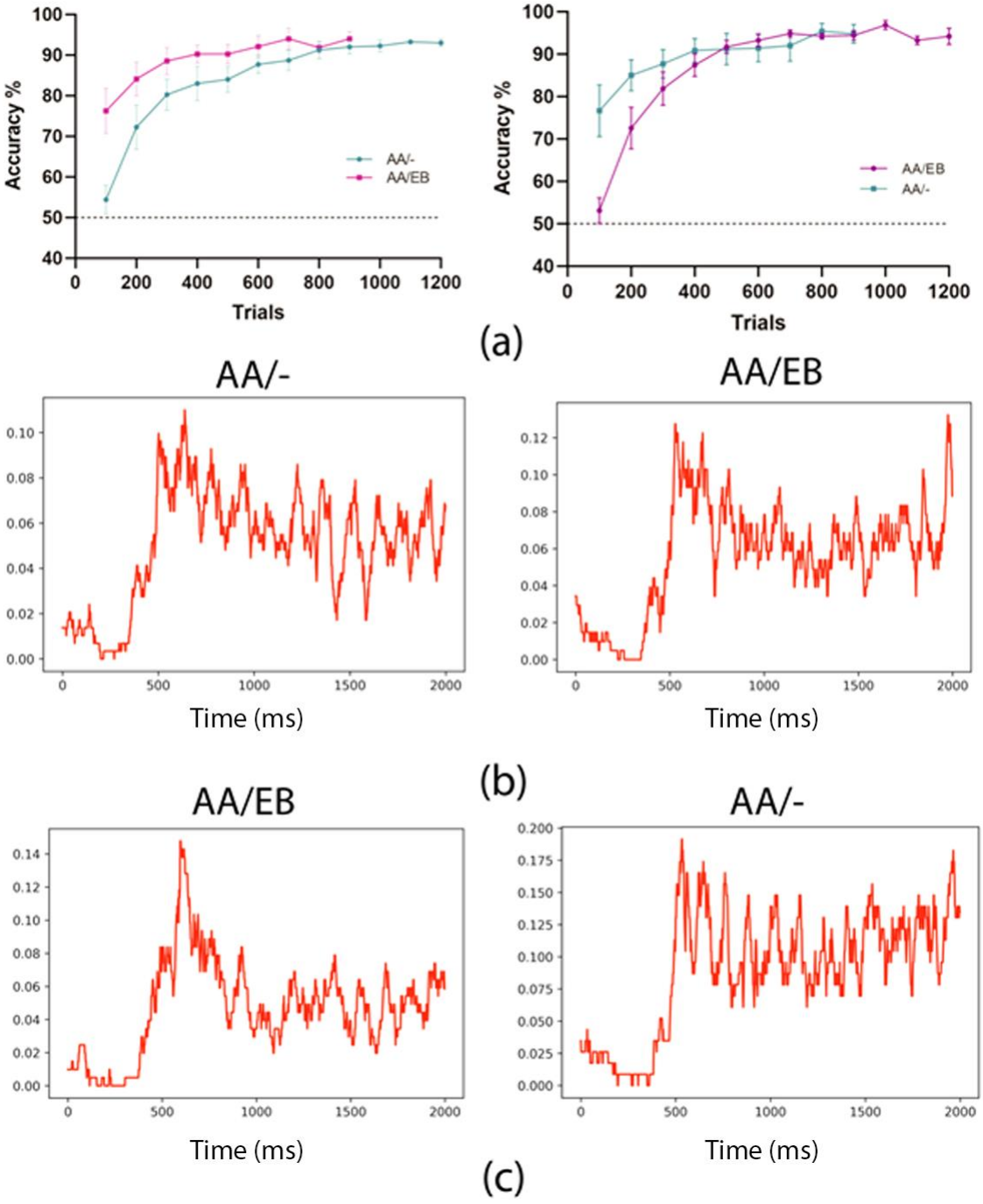


Figure 11: (a)Learning curves of animals performing AA/EB and AA/- in different orders shown by legend; (b) Avg. RT plot for set of animals performing AA/- followed by AA/EB (Grp1); (c) Avg. RT plot for set of animals performing AA/EB followed by AA/- (Grp2) [for (b) and (c), x-axis represents time in ms and y-axis represents avg. lick state of RTs]

Figure 11(a) shows the learning accuracies for a monomolecular odor discrimination task (odor pairs Iso-amyl Acetate (AA) and Ethyl Butyrate (EB) or mineral oil denoted by “-/blank interchangeably”). The average of the lick states for the RTs is also plotted along with. As can be seen, the same pattern is observed with the peak residing in the first half of the response time (2 seconds). Moreover, it appears that the decay of the peak for the case of AA/- where there was no odor for the non-rewarded trial there is a high level of overall noise. The peak in this case does not fall down to a similar baseline value.

The normalisation step involved is used to remove the influence of the number of RTs and preserve just the basic characteristics of the plot. So, for the analysis involving the quantification of the RTs, we compared them across different experimental frames. Fig. 12 shows the number of RTs for both the experimental groups across different odor discrimination tasks. There was no significant difference found across all the conditions.

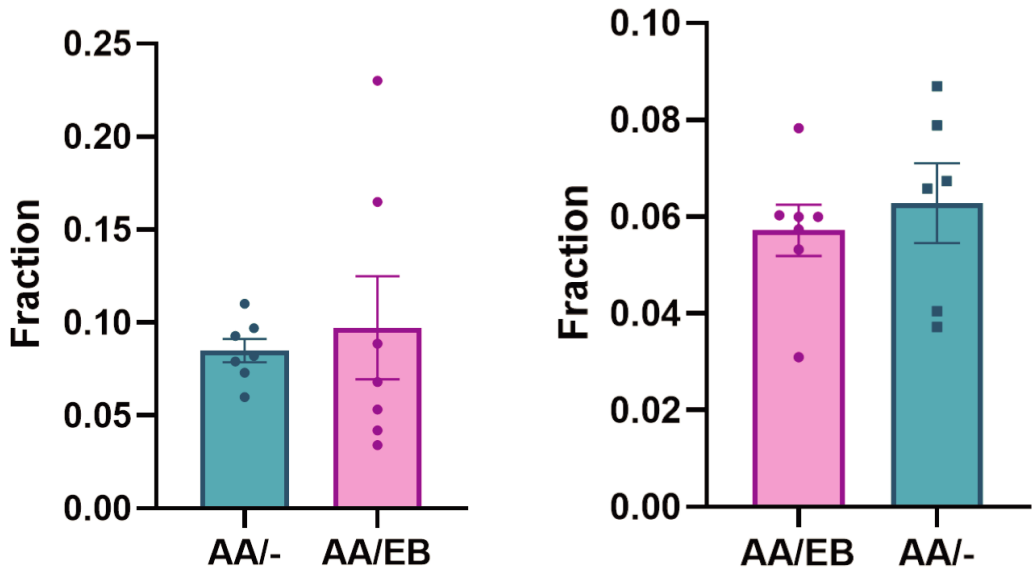


Figure 12: Fraction of RTs out of the total number of trials post data filtering step for 2 different experimental groups (Grp1 and Grp2) differing in their order of odor pairs; Teal green represents detection task and Pink represents discrimination task

Hence, we conclude that the RTs resulted in a similar curve with peak attributes similar to the ones obtained from previous experiments. Also relatively, the fraction of RTs out of the total number of trials considered for the analysis (>80% average accuracy trials) is consistent across different experimental groups.

3.3. DETERMINING THE REVERSAL WINDOW

In order to characterize the peak temporally, the same data of 1000 values (2000ms, each bin is 2ms in duration) was used to create a p-curve. *one-tailed t-test was carried out between the average of the first few bins considered to be the baseline (25 bins = 50ms) across all the subsequent bins in the total response window of 2 seconds. The window of the peak was determined by the point in time where the $p<0.05$ and $p>0.05$ respectively for the first time, giving a range for the window size on the temporal scale. This had to be a major inflexion point to be considered as the window duration.

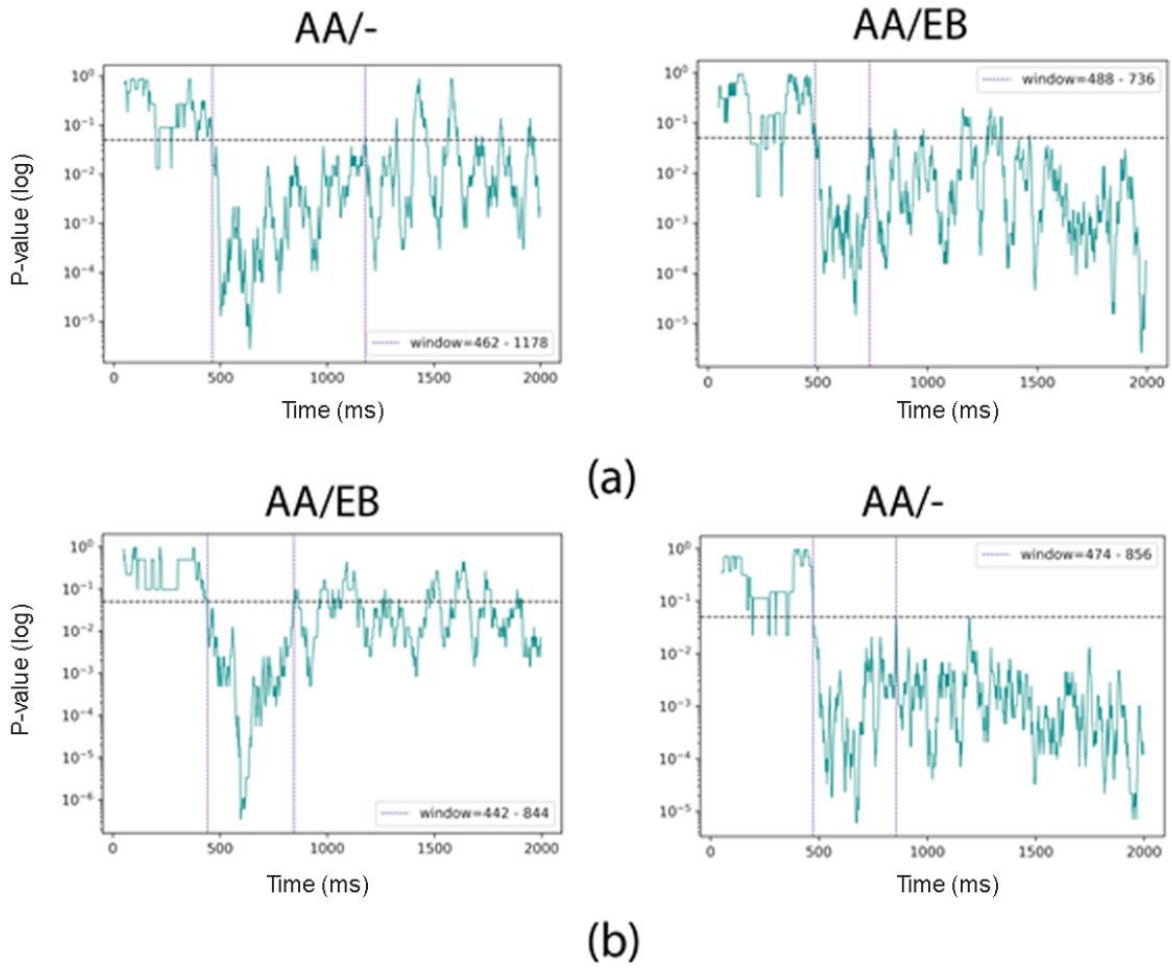
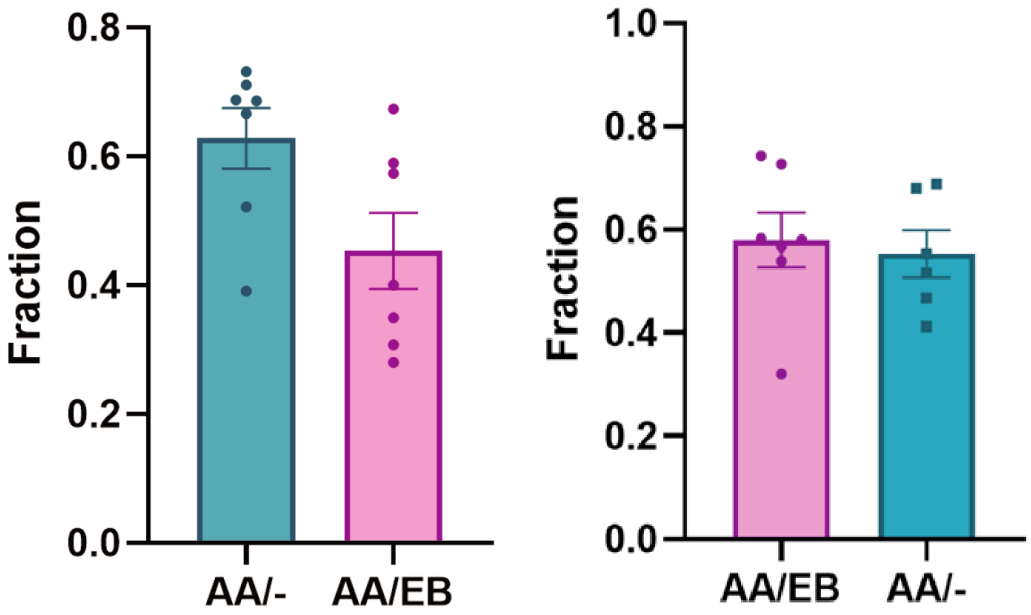


Figure 13: (a)p-curve for window determination of Grp1; (b)p-curve for window determination of Grp2 [x-axis has time in ms and y-axis has p-values in log-scale, horizontal line is for p=0.05]

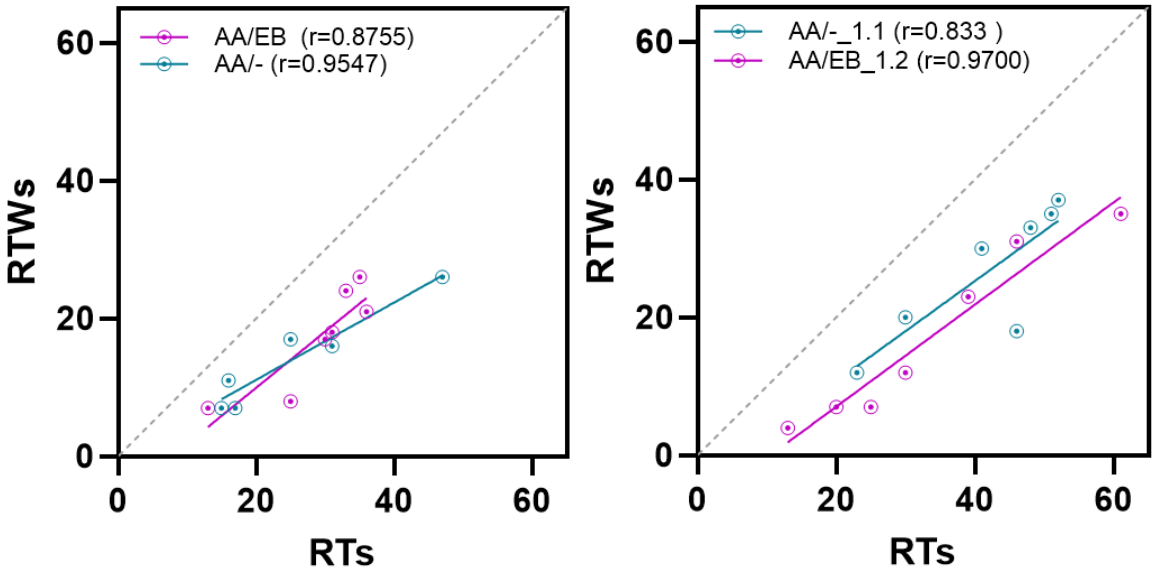
Fig. 13 shows the p-curves obtained from the analysis of the averaged lick states for the RTs. The window gives a probable range within which the majority of these

“decision reversals” take place. In order to quantify the proportion of the reversal trials that are responsible for this spike, we re-analysed the filtered trials and calculated the number of such trials that lay within the window range obtained for a particular experimental group. For all arbitrary window values, we took a default value of 1000ms as the window termination. This resulted in a subset of RTs that are confined to the ‘reversal window’ and hence were called reversal trials in the window (RTWs).

Fig. 14 shows the fraction of these trials out of the total reversal trials i.e. RTWs/RTs. They were not found to be significantly different across groups or experimental sets. Additionally, we also looked at the correlation between the number of RTs and the number of RTWs in order to determine the relevance of the window in the context of the experiment. The grey dashed line is for the line of identity which refers to maximum correlation. The purple and the green lines represent regression lines for the individual experiment groups. It was observed that, the value of Pearson’s r for all the sets lied within the range of 0.833 and 0.970. A high correlation signifies that the weightage of the RTs arises from these RTWs.



(a)



(b)

Figure 14: (a)Bar graphs showing reversal trials within the window (RTWs) for Grp1 and Grp2, whiskers: SEM; (b) Correlation plots with Pearson's r factor and regression lines for Grp1 and Grp2, circles represent single animals, grey dashed line represents ideal correlation

In the framework of a binary choice, when there is a reward associated with only one of the stimuli, animals tend to commit errors less frequently after having learned the task. Here, it is observed that even after learning, the animals tend to be fickle with their decisions. For example, in the case of the S- trial, they refrain from any licking ideally. However, in these so called RTs, they suddenly start licking and then quickly revert back to their non-licking decision. Hence, they are referred to as episodes of decision reversals and hence the name of reversal trials (RTs).

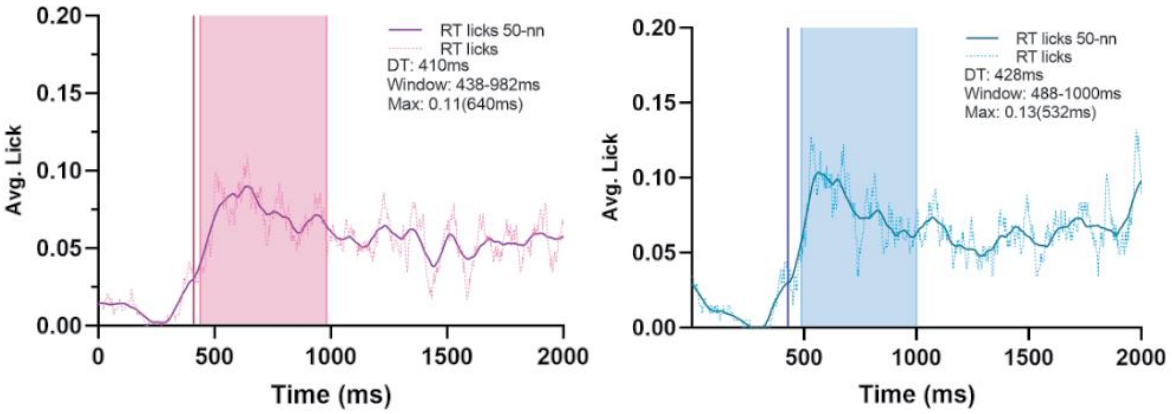
In this segment, we devised a method to determine a range of time in the response window for the reversal trials. This would be describing the temporal attributes of the RTs. We also defined a new parameter called the reversal trials in the window (RTWs) in order to emphasise on the subset of these reversal trials that are confined in a small duration of the response window, approx. 400-600ms out of 2seconds i.e. 20-30%, called as the reversal window. Roughly 60% of the reversal trials lie in this small segment (some cases as high as 85-90%). This point was reinforced with the help of

a correlation plot between the RTs and the RTWs. A high Pearson's r value confirms the significance of the reversal window across different experiments conducted.

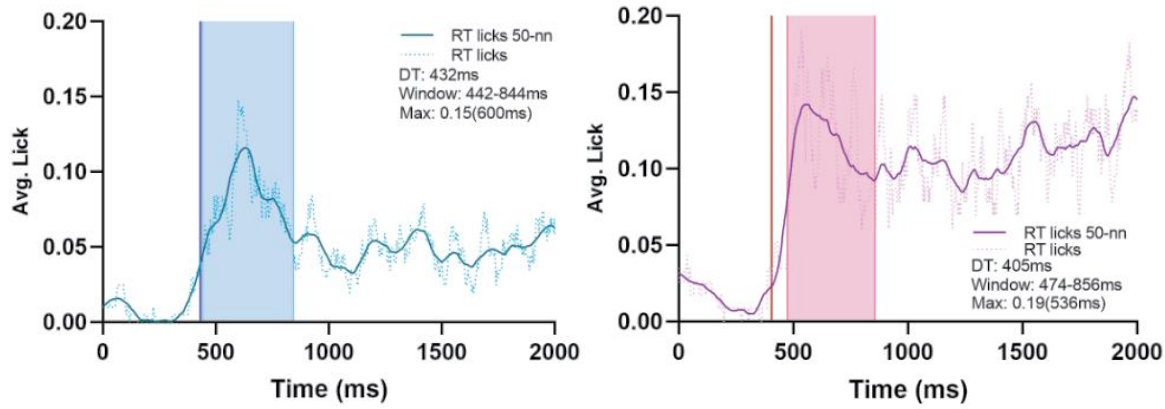
3.4. Effect of training sequence on reversal trials

Fig 15 shows the average lick plots of the reversal trials (dashed lines) and the same plots with a smoothing factor of 50 (50 nearest neighbours i.e. 5% of data points). This is just for purpose of representation. The statistics, t-tests and calculations of other characteristics are determined on the original data. The plot also includes the average of discrimination times (discrimination time or DT is calculated by taking S+ and S trials 150 each, and then running a one-tailed t-test across rewarded and the non-rewarded trials bin wise hence getting 1000 values. The point where they significantly differ i.e. the last p-value <0.05 determines the discrimination time for that animal).

It was found that the peak was much more pronounced for the AA/EB case compared to AA/- which was contrary to our original hypothesis. It had a proper peak and a lower basal noise for post-peak decay. The rubric behind designing such a paradigm was to create a relatively easier task for the animals (AA/-) with only one stimulus, hence the task was similar to a detection task. This was then followed by a standard simple odor discrimination task (AA/EB), which was thought to be the harder task for the animal. Although the number of RTs is not differing significantly, the post decay noise and a relatively flat peak in the former case suggests that animals were more confused while performing the discrimination task involving a single odor for the stimuli.



(a)



(b)

Figure 15: Reversal plots for Grp1 (a) and Grp2 (b) with dashed lines representing mean of the lick states and bold lines represent K=50 smoothed curve. Reversal window is shaded with vertical coloured line marking the Discrimination time [Pink represent AA/- and blue represents AA/EB, plots follow the chronology of experiments in each group]

When order of the experiments was reversed i.e. a different set of animals were trained on AA/EB first followed by AA/-, a similar pattern was observed. It was observed, just like before, a sharper and more refined peak was observed for the case of AA/EB and for AA/- the peak had much more post-decay noise as can be seen in Fig. 15(b).

Fig 16 shows the effect of chronology of olfactory training on the resultant decision reversal plots. These are the same plots as shown earlier, smoothed with 50 nearest neighbours and overlapped for each experimental group. As, can be clearly seen, AA/EB shows up somewhat similar in both the cases i.e. whether it was tried on naive set of animals (b) or on a set of animals that have already been trained to an odor discrimination task (a). Both the cases, yield a curve with a sharp peak and a slow decay. However, in the case of reversal trials for AA/-, when it was performed on a set of naive animals, the peak was diluted and reduced. Also, the peak decay was very slow and noisy, resulting in a wider peak. In the experimental group where it was the second odor pair (b), it can be seen, that the overall peak value is much higher, however the decay is almost obtuse. Post peak noise is very prominent despite the fact that animals are well-accustomed with the paradigm as they have already finished one odor-discrimination experiment.

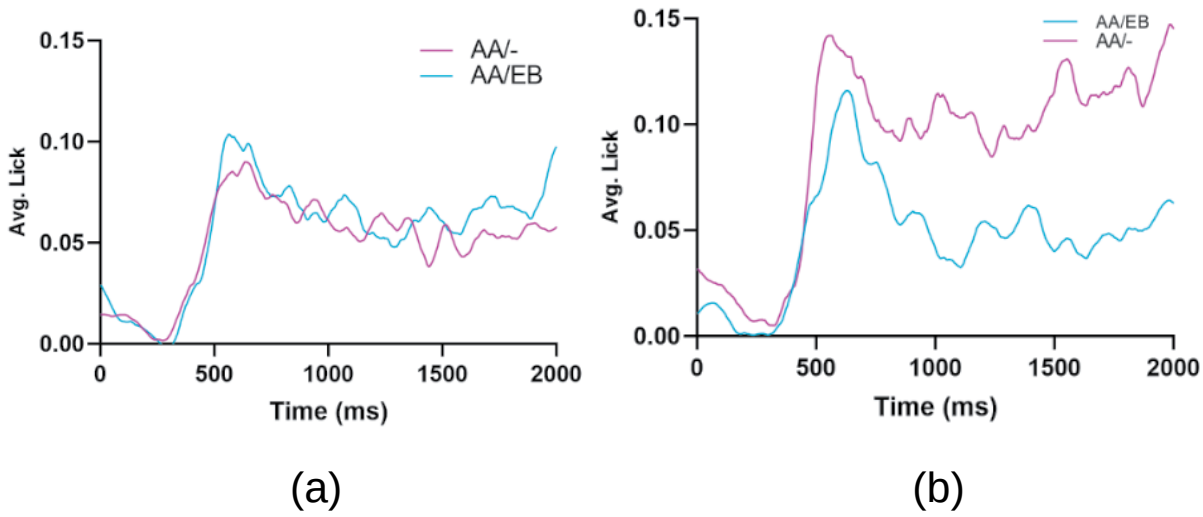
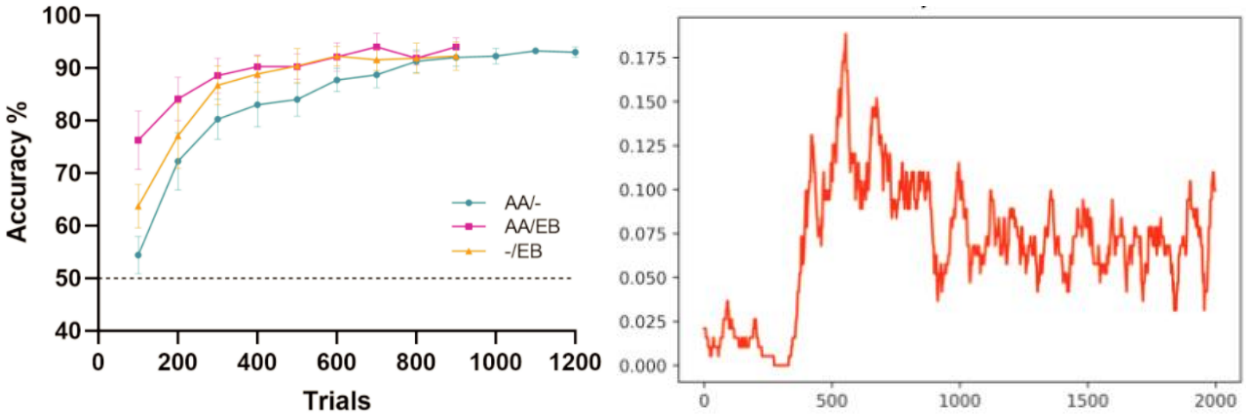


Figure 16: Overlapped plots of K=50 smoothed curves derived from RTs of individual groups- (a) for Grp1 and (b) for Grp2

We designed these paradigm, so as to provide the animal with a contextually easy experiment in one group (AA/- followed by AA/EB) and a harder experiment in the other (AA/EB followed by AA/-). We hypothesised, that reducing the complexity of the task might make animals attain lower episodes of confusion/dilemma once they have learnt the experiment. If that would have been the case, we would have observed a sharper peak and a finer post-peak decay. However, dampening of one of the odor, irrespective of the associated reward contingencies in a discrimination task resulted in more obscurity and confusion. Hence, reversal trials for AA/- task constitutes dispersed lick responses throughout the stimulus duration instead of the confinement observed for the case of AA/EB. A possible explanation to this anomaly is that, not having an odor for the stimulus actually challenges the detection and discrimination abilities of the animals. For our analysis we have focused on the non-rewarded trials only, and keeping the diluent as the S- stimulus could be leading to such a result.

3.5. SIGNIFICANCE OF PRESENCE OF ODOR AS NON-REWARDED STIMULUS

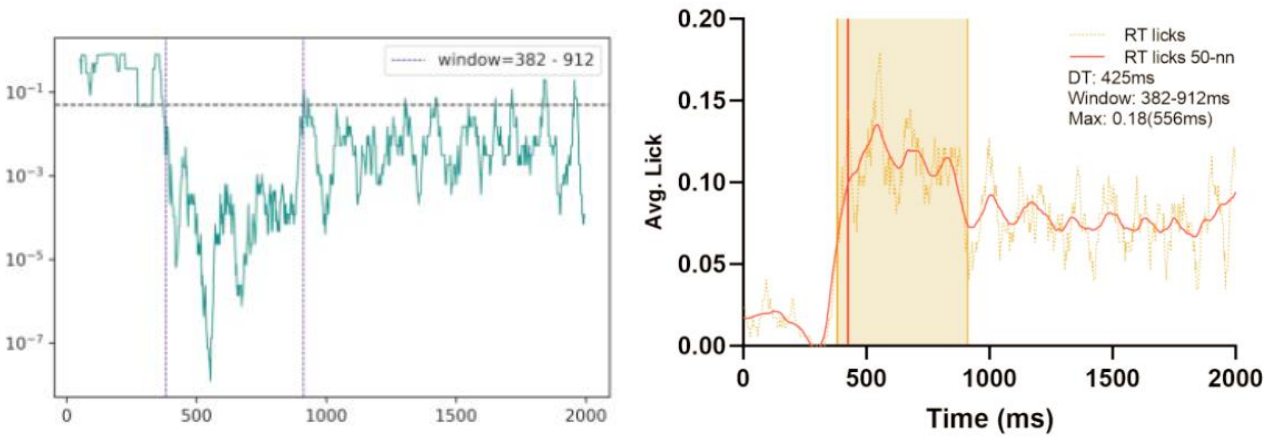
In order to confirm our hypothesis, we further designed an odor pair discrimination task with the animals that have already performed AA/- and AA/EB in the order to train on -/EB, i.e. just the mineral oil (diluent used for odors) for the S+ stimulus and EB (1% odor diluted in mineral oil) for the S- stimulus. Fig 17 shows the learning curve with respect to the previous learning curves. The order of training was: AA/-, AA/EB, -/EB. It also shows the average of the reversal trial lick states repeating the similar pattern as seen earlier.



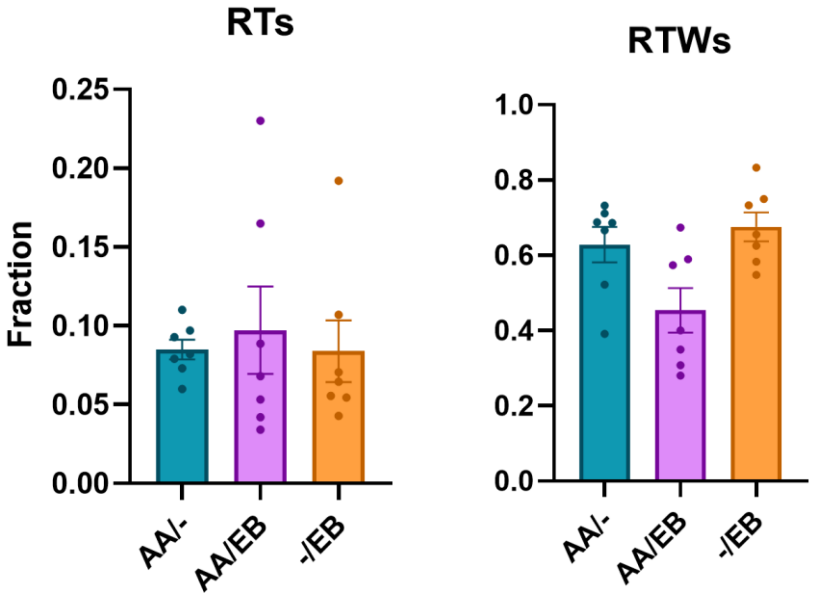
(a) (b)

Figure 17:(a) Updated learning curve with Grp1 animals performing -/EB after AA/- and AA/EB; (b) Avg. lick state of the RTs for Grp1 animals in case of -/EB [x-axis has time in ms and y-axis has avg. lick state]

Fig. 18 shows the p-curve obtained after performing the t-test, yielding the window range for the RTWs. The number of RTs is found to lie in the same range as before and so is the case for the RTWs. Hence, this does not make an exception to the general pattern of reversal trials and the reversal window.



(a) (b)



(c)

Figure 18:(a) P-curve of the RTs for -/EB for Grp1 animals [x-axis has time in ms and y-axis has p-values in log-scale, horizontal line is for p=0.05]; (b) Avg. lick plot with K=50 smoothed curve, DT and reversal window, (c) Updated fraction of RTs and RTWs as bar graphs with -/EB included for Grp1 animals

However, as is seen in Fig 19, when we observe the smoothed out curve for the reversal trials in comparison to the previous experiments, it can be clearly seen that the post-peak decay for -/EB behaves similar to that AA/EB. Hence justifying the fact

that a noisy pattern is observed only in those cases when stimulus intensity dampens for the non-rewarded trial (S- having just the diluent).

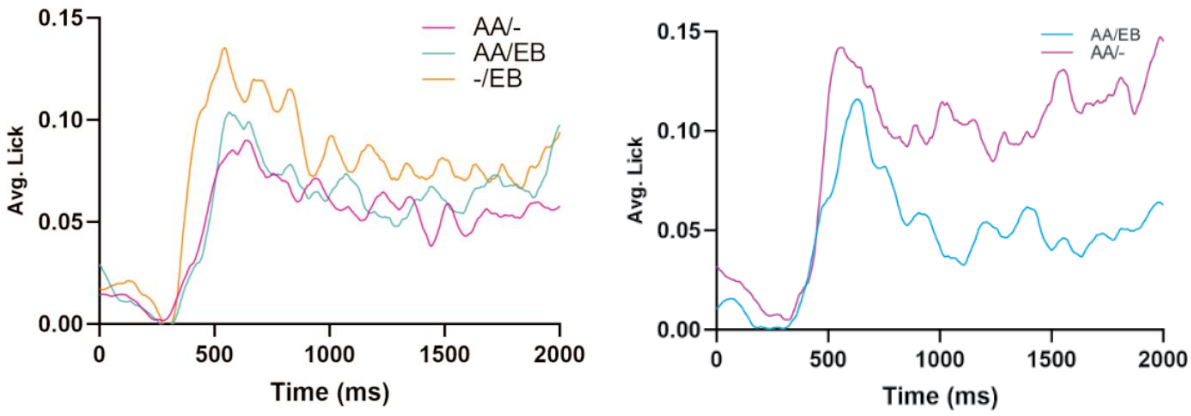
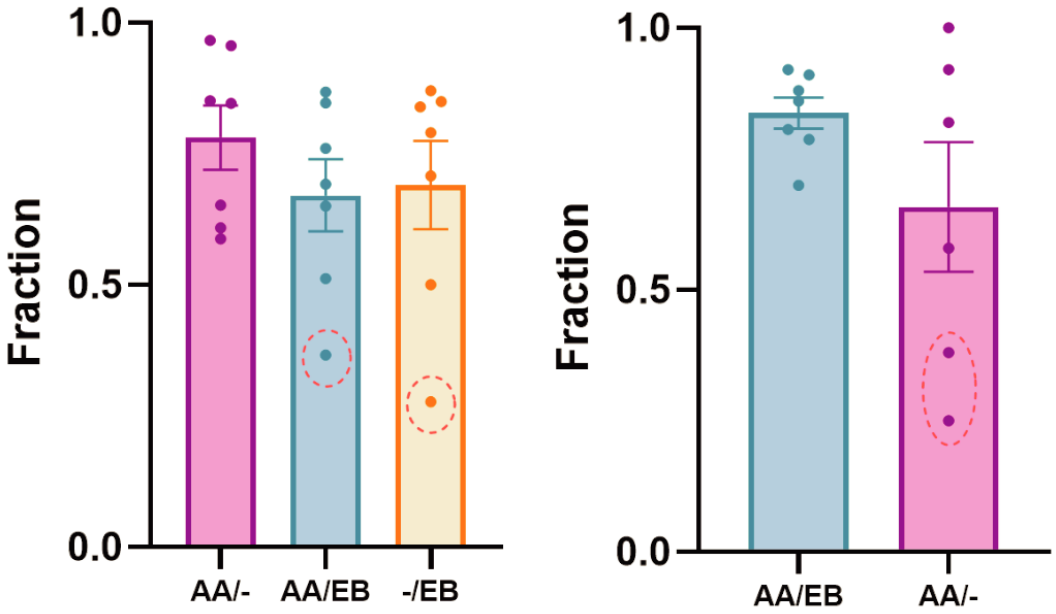
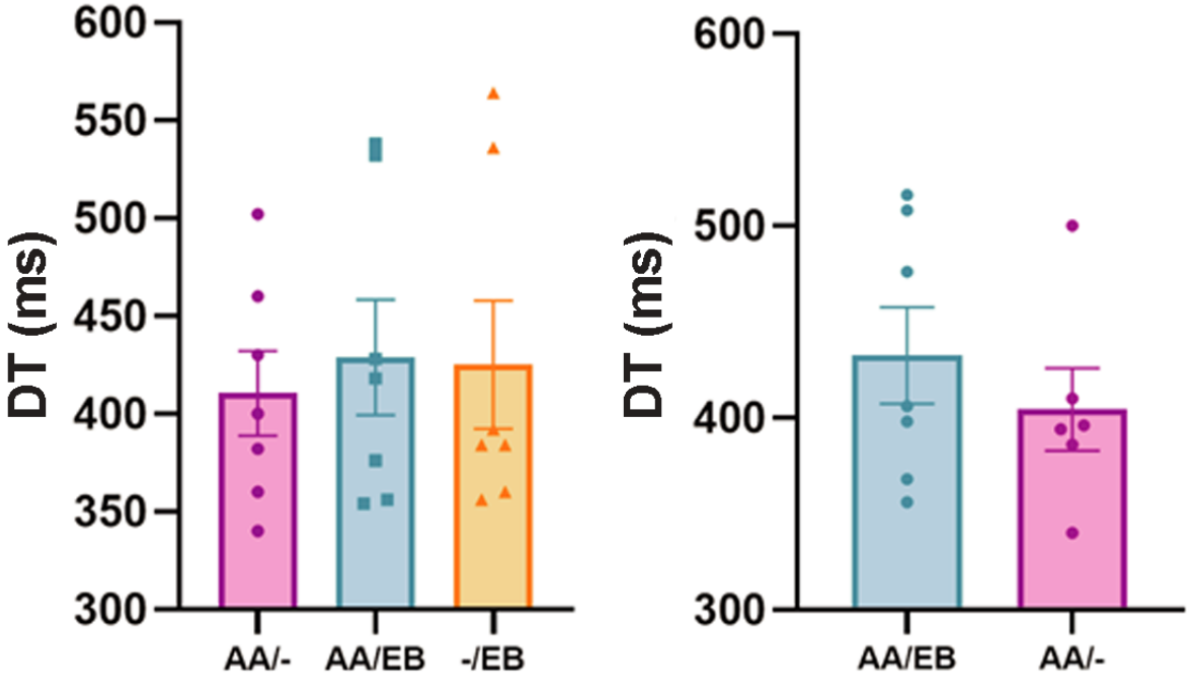


Figure 19: Updated smoothed RT plots for comparison between Grp1(left) and Grp2(right) with -/EB data included

Another observation made during the analysis is that, in each group some of the animals were over motivated. This resulted in a lot of incorrect frequent-licking trials in the final set of reversal trials. In order to resolve this issue, we looked at the fraction of correct trials among the RTs and the final analysis involved only those animals that had a >50% correct rate for reversal trials. The animals marked with red dashed ellipses in Fig 20 were such animals that had a <50% of correct response rate and hence were not included in the final analysis. This also shows, that DT calculated for all these animals were comparable across groups across odor pairs. Hence, there was not inherent difference in the animals regarding the discriminability between odors (or in some cases between odor and diluent).



(a)



(b)

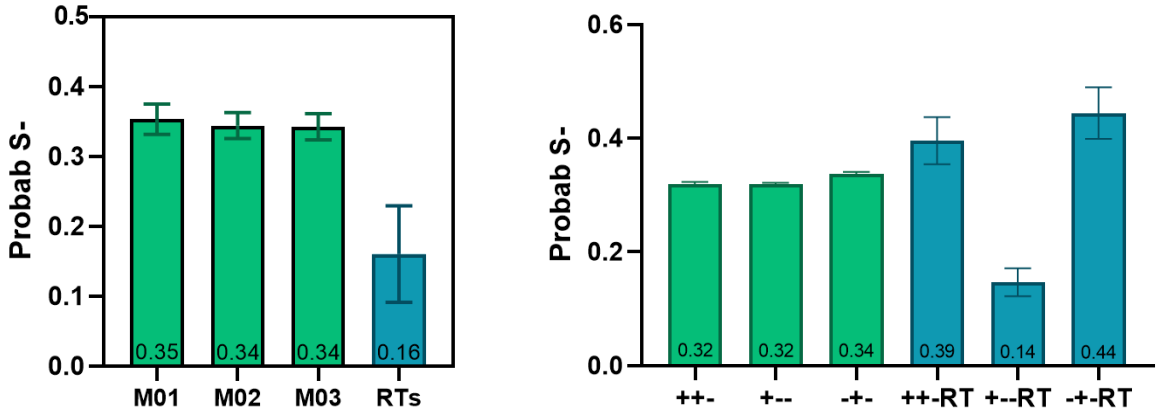
Figure 20:(a) Bar graphs depicting fraction of correct trials among the RTs; (b) Bar graphs comparing DTs across different odour pairs for both Grp1 and Grp2

Thus, alleviating either of the stimulus in an odor discrimination task actually emerges as a more complex task for the animal. This was reverted by shifting the odor-less cue

to the S+ stimulus while bringing in an odor stimulus (EB) for the S- trial while still preserving the context as the animals were performed to do AA/EB before. Our analysis shows that animal exhibits similar lick responses when there is a prominent odor present as non-rewarded stimulus i.e. lick responses in -/EB was similar to that of AA/EB.

3.6. Effect of pseudo-randomisation on occurrence of revision trials

As mentioned earlier, the computer creates a pseudo-randomised list of trials for every block of 20 trials; it being pseudo-randomised such that there are no occurrences of a tuple of three S+ or S- trials. We wanted to see how occurrences of RTs is affected by the kind of trials (rewarded/non-rewarded) preceding the reversal trials. We started with a pair of two consecutive S- trials ("-,-" where '-' refers to a S- trial and '+' refers to a S+ trial). First the probability of having an S- trial preceding to a randomly sampled S- trial was calculated based on a simulation prepared using custom-written python scripts. This basically gave the probability of getting a pair of S- trials (-,-) coming up entirely from the pseudo-randomisation of the computer code responsible for creating the blocks of trials. All the sampled S- trials were from blocks that had an average accuracy of >=80% so as to keep it in lines parallel to the criteria involved in the calculation of the RTs. This was then repeated in 3 different animals so as to confirm the result. Following this, all the reversal trials for every animal in the training set AA/EB (n=7) were used to calculate the probability of having an S- trial preceding to a RT (all reversal trials are S- in nature). In other words, this gives the probability of getting a reversal trial in the second position in an S- pair (-,-).



(a) (b)

Figure 21: S- probability bar graphs for (-,-) pair (a) and all possible tuples of 3 (b)[green represents data from randomly sampled trials, dark blue represents data from RTs pooled over multiple animals of the same group]

Fig 21 shows that there is a significant difference in the case of RTs. In a pair of (-,-) trials, the probability to get a RT is relatively lower. Further, we also looked at similar simulations and probability calculations in the case of tuples of 3 trials. Since, the conditions of pseudo-randomisations removes the possibilities of (+,+,+) and (-,-,-) trials, 6 other possible combinations are left, out of which only 3 have a S- trial at the last position: (+,+,-), (+,-,-) and (-,+,-).

We looked at the relative probability of getting these tuples in a randomly sampled set of S- trials (tracing back to 2 trials this time) which is shown in Fig. 21(b). On taking the RTs and tracing back to 2 trials to find the probabilities of the same tuples, we observed, that the chances of getting an RT at the last position of a tuple in the form of (+,-,-) was relatively lower. This is in sync with results obtained earlier with respect to (-,-) pair. It is observed that whenever, if a random reversal trial were to be sampled, the probability that it would follow a consecutive pair of S- trials would be very low.

4. DISCUSSION

An efficient and timely reversal of wrong decisions can lead to an enhanced accuracy ad positive reinforcement. According to research done on human subjects, changes done to an intial decision made better corrections to an error more often (Berg et. al., 2016). A study by Berg and colleagues used behavioural data and visually guided perceptual decision-making tasks to develop a computational model that could explain decision mechanisms and correct bad decisions. Studies on rodents and humans suggested that the decision-making process involves a speed-accuracy tradeoff. Individuals' tendency to make decisions more quickly or hurriedly, which could compromise the decision's accuracy, is known as the speed-accuracy tradeoff (Wang et. al., 2002). This phenomenon may explain the impulsive decision-making process seen in numerous neuropsychiatric illnesses, which results in more wrong decisions. In a study by Carpenter and the group using human participants, it was found that accuracy was affected when subjects were asked to make decisions quickly. According to reports, quick responses fell short of accuracy (Reddi et. al., 2000) in most cases. Another study by Rinberg and colleagues focused on the speed-accuracy trade-off that occurs in rodent olfactory guided activities. In a two-alternative choice task, the duration of the stimulus was manually adjusted to examine the relationship between decision accuracy and stimulus duration. They created the paradigm with improved control over the complexity and duration of the stimulus. The findings suggested a direct relationship between stimulus duration and the accuracy of the decisions being made by the animal with increasing stimulus complexity, i.e., that complex odour mixtures required animals to sampl for longer periods of time than simple odours in order to make the correct decisions. Additionally, the likelihood that quick responses would be accurate was surprisingly low (Rinberg et. al., 2006, Chittka et. al., 2009). This suggests that there is a greater tendency for poor decisions when the situation calls for a speedy response. Perhaps the most intriguing question is, "What happens next?" Do bad choices get corrected? Does the adaptability of cognitive processes allow for changes after an action has been taken? Does the presence or absence of stimulus have an impact on these early reactions and reversals as well?

We attempted to investigate stopping/reversal behaviour observed specifically in the context of non-rewarded trial in an odor discrimination task. Mice were trained in a head restrained setup to distinguish between two different odors in a Go/No-go paradigm. They were water restrained and received water as reward for the rewarded trial when they fulfil a particular lick criteria which is pre-determined by the experimenter. Initially, animals lick for both the odors resulting in an average accuracy at chance level (~50%). As the training progresses, animals start to associate stimulus with the reward and learn to refrain from licking for the stimulus the non-rewarded trial. The animals get no feedback about their performance towards the S- trials since there is no negative reinforcement (for example some experiments indulge in providing a mild foot-shock for every wrong trial).

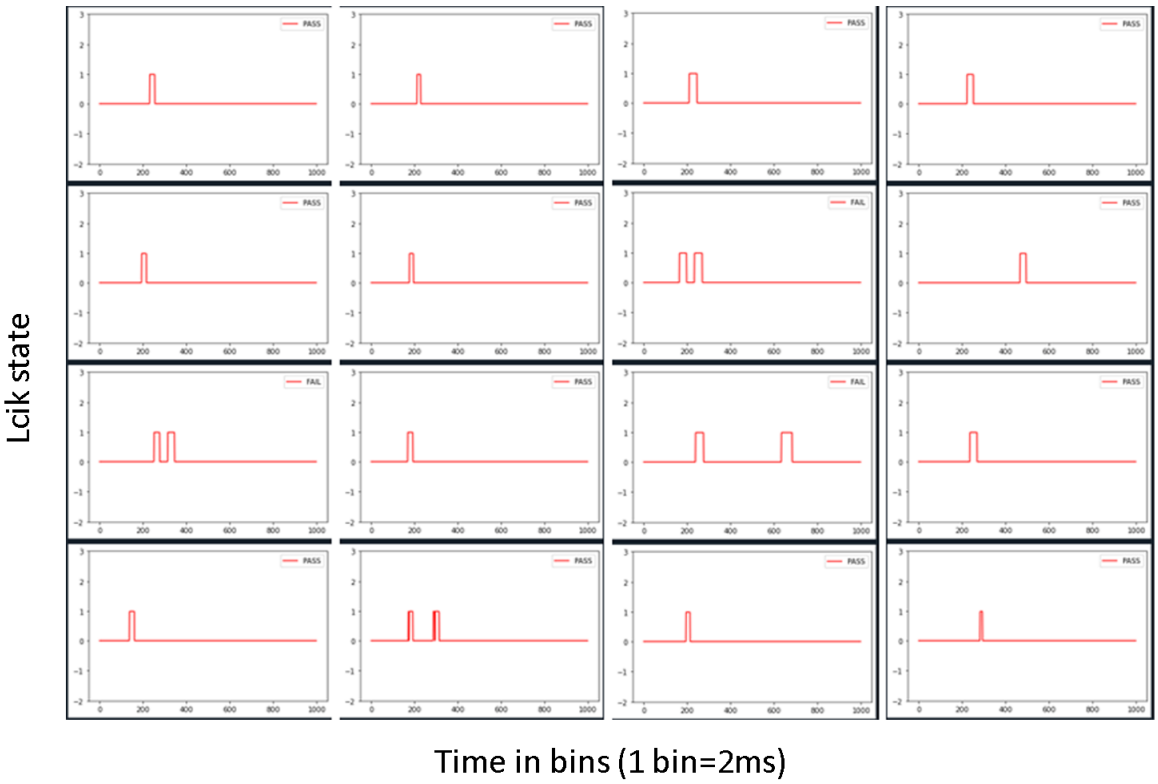


Figure 22: Compilation of the majority kind of RTs, all correct S- trials, recorded during the data filtering process (x-axis has time in bins; 1bin=2ms and y-axis shows lick states as 1 or 0)

However, we have seen, even after the animals have learnt and perform at a very high average accuracy, tend to show this peculiar behaviour of lick spikes (mostly 1-2 licks) and these are filtered out at the end known as the reversal trials.

Fig 22 shows a collection of all such trials accumulated from a single animal in one of the data acquisition steps. It is clear that a major chunk of the final peak that is arrived at is due to these individual lick responses registered by the animals. To emphasise the point this particular pattern has been seen repeatedly. Fig 18 shows a summary of RTWs from different experiments done in the lab previously. Although the questions that being addressed were very different in each of these experiments, the presence of decision reversal trials, the pattern of confined decision reversals yielding in the window formation and the factor of RTWs still holds true.

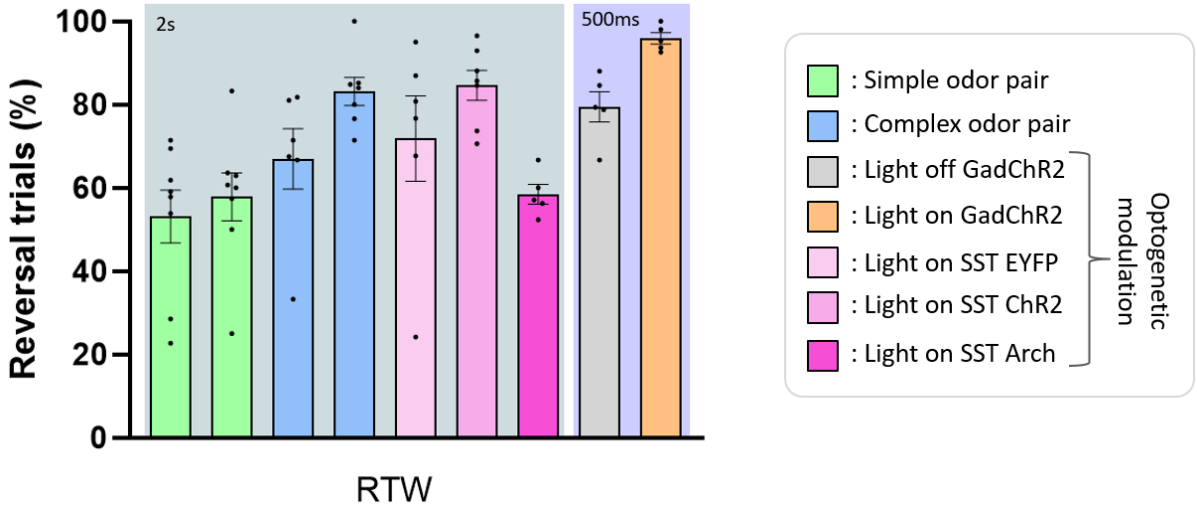


Figure 23: Compilation of RTW bar graphs across different experimental groups

Similarly, if we pool in data from all these previous experiments and plot a correlation matrix between the RTs and the RTWs to get a generalisation of all the decision reversal windows and the number RTs and corresponding RTWs for individual animals, we get a correlation plot as shown in Fig 24. It shows a very high correlation with a Pearson's r value of 0.95 and an R squared value of 0.90. This data is from over 20 different odor discrimination experiments involving decision reversal data from more than 140 animals with majority lying within the range of 10-30 trials. Although the data spreads out quite a bit as it goes past 60 RTs, the pattern of correlation still holds.

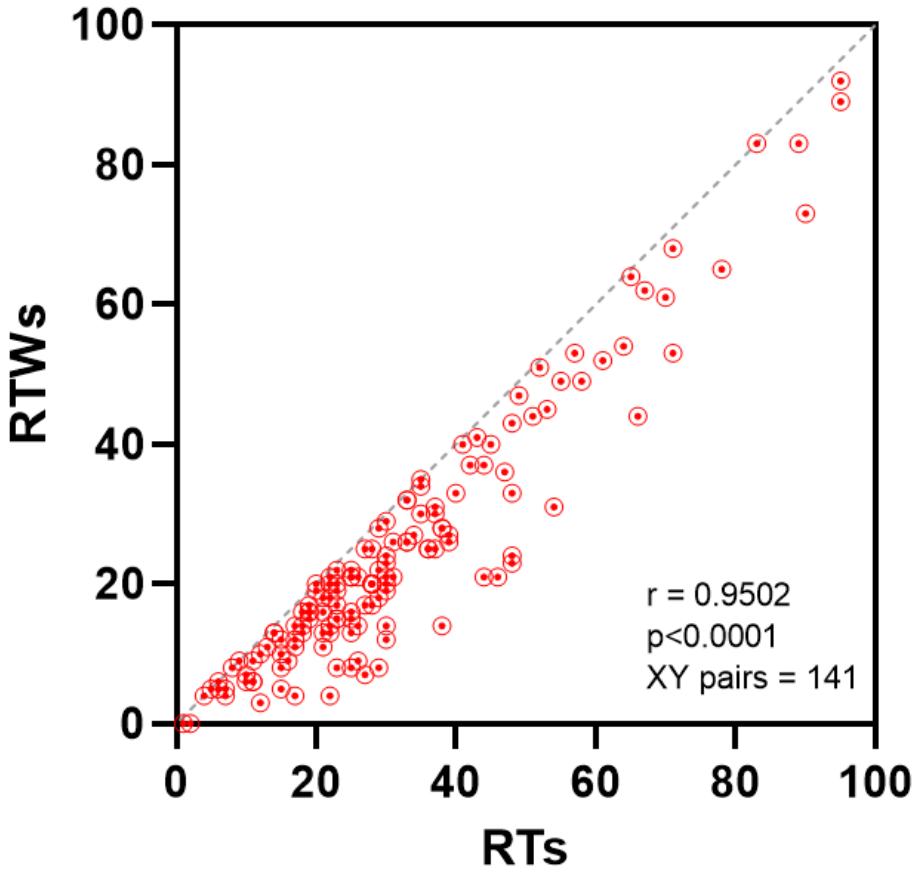


Figure 24: Correlation plot of all animals across different experimental groups pooled together to look at RTs at a population level

Another recurring feature in these reversal plots was that it always represented a lognormal distribution, some of them fit with a squared value of 0.80 or above. A lognormal distribution, according to probability theory is one in which the data represents a continuous probability distribution of a random variable whose natural logarithm is naturally distributed. For example, if 'X' is log-normally distributed, then 'Y=ln(X)' has a normal distribution. It is generally used in engineering and medicine to represent a distribution that is skewed to the right, has a smaller mean and a high variance. Interestingly, occurrence of log-normal distribution is not new in biological scenarios. In neuroscience itself, distribution of neuron firing rates across a given population is log-normal in nature and so is the distribution of synaptic weights. With intervention at the right time causing its adequate control, a highly communicable epidemic also shows properties of lognormal distributions in the hospitalised cases.

For example, the amount of time spent by an individual on an online

advertisement/articles follows a lognormal distribution and so does the average length of a chess game as shown in Fig 25.

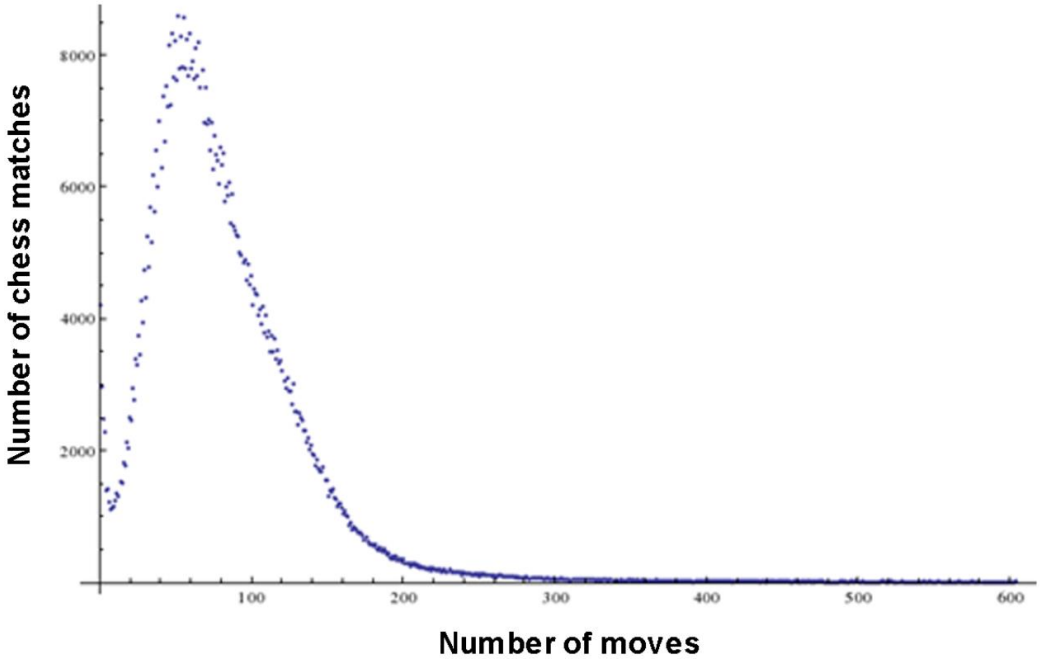


Figure 25: Example of log-normal distribution (x-axis has length of moves before check-mate and y-axis has number of games recorded)

It was also observed from the RT analysis from experiments done in the lab before is the consistent window range. Fig 26 shows the initial and final points of the reversal window obtained by doing the t-test on the average of the lick states for the RTs. The average values are 352ms and 861ms respectively, with a high variance for the window out value.

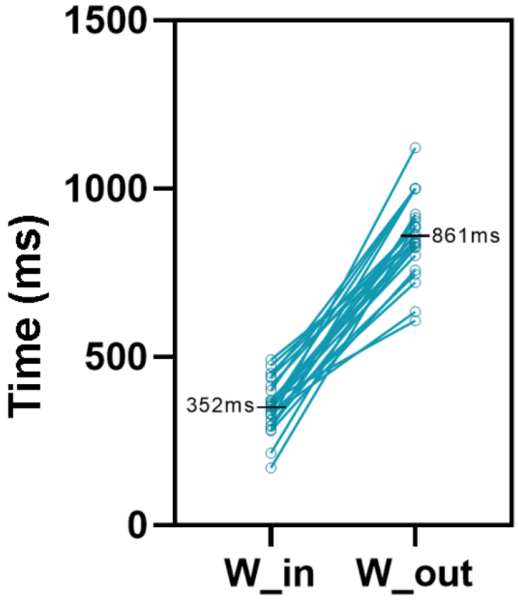


Figure 26: Depicts the duration of reversal window over all animal groups across different experiments (W_in and W_out are for window begin and window end)

The ending of the window based solely on the p-curve could be inconclusive at times, which is quite intuitive since the post-peak decay period is highly prone to have noise and that could mess up with the p-curve derived value to represent the window ending. Hence, many a times, when the average of the RT licks was noisy enough to not yield a window range that was realistic in terms of the window ending, a default value of 1000ms (1 second) was considered as that was a good approximation for the (avg. + 2*SD) value.

By performing various odor discrimination experiments, it was also observed that the absence of odor in the non-rewarded trial turned the task more complex i.e. animals found it more confusing to have to distinguish between an odor mixed with an apparently odor-less solvent (or odor-diluent) rather than discriminating between 2 different odors altogether (respectively mixed in solvent). A possible explanation for this behaviour is that, since there is no odor present in the solution, there is a distinctive lowering in the attention, hence the occurrence of the errors/decision reversals are more spread out throughout the response window mimicking somewhat of a random distribution rather than a confined window formation.

Another interesting point to note here is that, although the parameters of the curve remain mostly uniform, recent experiments performed in the lab have shown that the number of reversal trials are affected by the complexity of the task. Fig 22 shows how with increasing the complexity of the experiments progressively, the number of RTs tends to increase progressively which makes sense since, more complex the task in hand, higher would be the frequency of getting confused. This opens up further means for explaining the nature of these reversal trials. We can hence aim at modulating the nature of this behaviour by playing around with different parameters in a standard odor discrimination task, to begin with: complexity of the experiment.

Thus, as a part of the thesis, an attempt was made to establish a robust behavioural

parameter towards non-rewarded trials and its attributes such as the average lick states, curve characteristics, window duration, and reversal trials in the window. It signifies the presence of an odor stimulus in the context of non-rewarded trials and how the pattern of RTs changes once the stimulus is devoid of odor. The general property of reversal trials is consistent across different experimental conditions however, different characteristics of this behavioural phenomena could be altered by modifying stimulus properties i.e. increase/decrease in stimulus complexity/duration. This study opens up avenues for further investigation in the domains of decision reversals in an olfactory discrimination context. Moreover, currently running experiments in lab are trying to look at how this behaviour is influenced by the introducing a delay in reward period, increasing the stimulus complexities further in wild type as well as in stress and disease models of mice.

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