

Appendix 2:

IBL protocol for mice training

Table of contents

Introduction	3
Psychometric curve metrics	3
Training stage description	5
Habituation	5
Training	5
Training stage timeline	7
Outline	7
Surgery and Water restriction timeline	7
Habituation timeline	7
Signs of stress during head-fixation	8
Training timeline	8
Criterion	9
Criterion to assess learning	9
Level 1	9
Not trainable	9
Level 2	9
Task parameters	11
Visual stimulus	11
Onset tone	11
Negative feedback tone	11
Reward	11
Trial side proportions	12
Timing	12
References	12

Introduction

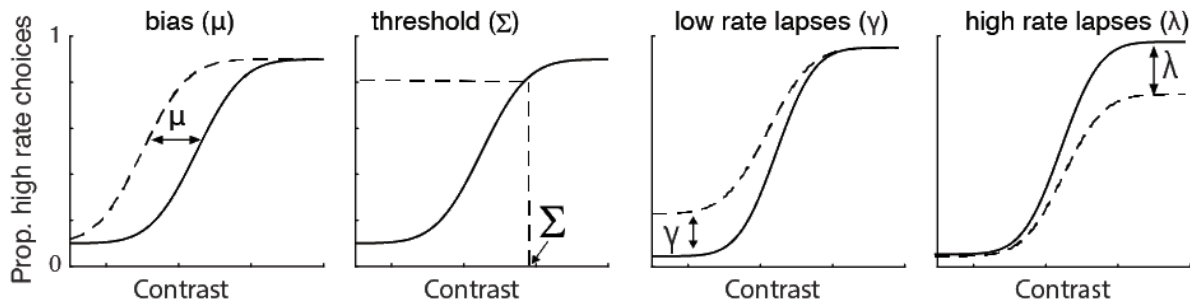
Mice are trained in a rig consisting of a screen and a steering wheel. They are trained on a 2-alternative forced-choice task requiring them to turn the wheel in order to move a visual stimulus (a Gabor patch) from the periphery to the centre of the mouse's visual field (see Figure 1A in *International Brain Laboratory et al., 2019*).

Mice go through three training protocols :

- `habituationWorld` during which visual stimuli are moved on the screen automatically.
- `trainingChoiceWorld` during which visual stimuli have to be actively moved by the mouse; left and right stimuli are presented with equal probability (50/50).
- `biasedChoiceWorld` during which visual stimuli have to be actively moved by the mouse; left and right stimuli are presented with different probability in blocks of trials (20/80 and 80/20).

Several contrasts are presented (from 100% to 0%) depending on the task level. Psychometric curves can be computed for the three different types of trial block: 50/50 (see Figure 1F in *International Brain Laboratory et al., 2019*), 20/80 and 80/20 (see Figure 4B in *International Brain Laboratory et al., 2019*).

Psychometric curve metrics



The psychometric curve is determined by:

$$\gamma + (1 - \gamma - \lambda) * (\text{erf}((xx - \mu) / \Sigma) + 1) / 2 \quad (\text{Code link})$$

Where

`xx` is a vector of contrast values

μ is the bias

Σ is the threshold (slope)

γ is the low lapse rate

λ is the high lapse rate

Training stage description

Habituation

On the two days before head-fixation the mice are handled for at least 10 minutes and given water from the hand. On the second day the mouse is also allowed to freely explore the training rig for 10 minutes.

After this the mice are habituated for three days, headfixed in the rig with the choice wheel fixed. Stimuli are presented at random initial locations (+ or -35 degrees in the azimuth, 0 degrees elevation relative to the center of the mouse's visual field) for an average of 10 seconds (drawn from a normal distribution with a standard deviation of 2 s). The stimulus then appears in the centre of the screen and 500ms after, a 3ul reward is given. The stimulus stays in the centre for a total of 1 second before disappears. A one second period of grey screen follows before the next stimulus. This stimulus is a full-contrast Gabor patch, comprised of a sinusoid with a spatial frequency of 1/10th of a cycle per visual degree masked by a Gaussian window of 7 square degrees. The spatial phase of the stimulus is randomized each trial, while the orientation angle is kept constant (0 degree, vertical bars). The mice are headfixed for 15-20 minutes on the first day, then 20-40 minutes the second day, and for 60 minutes the third day.

Training

On the fourth day the mice are headfixed with the choice wheel free and begin the 'active' phase of training. The same stimulus is used, but the contrast is randomly chosen from a set of contrasts each trial. Initially, this set comprises 50% and 100% contrasts. The mouse must perform a series of trials where it moves the stimulus from the initial position to the center of the screen to obtain a water reward. At the beginning of each trial, the mouse must not move the wheel for a fixed period for the trial to continue. The length of this period is between 200ms and 500ms, with the lengths drawn from an exponential distribution with a mean of 350ms. If the wheel moves during this period the timer is reset (note that the period length is kept constant during a given trial). If the mouse does not move the wheel during this period, the stimulus appears on either the left or right (- or +35 deg) and an onset tone is played for 100ms. The tone consists of a 5 kHz sine wave with a 10ms ramp duration to prevent onset artefacts.

As soon as the stimulus appears the mouse has 60 seconds to move the stimulus to the center of the screen (0 deg) to receive a 3ul reward or 35 deg further to the periphery, causing an error timeout. Initially, the stimulus moves 8 visual degrees per millimetre of movement at the wheel surface. If the mouse completes at least 200 trials within a session, the gain is immediately halved. Then the wheel gain remains at 4 visual degrees per millimetre for all future sessions. On incorrect responses (movement greater than 70 degrees), or if the mouse fails to reach

either threshold within the 60 second window, the stimulus is fixed at 70 degrees azimuth for 2 seconds on the side the stimulus originated and a noise burst is played for half a second. After this the stimulus vanishes and the next trial begins.

If the response was incorrect and the contrast was 'easy' ($\geq 50\%$) then the following trial is classified as a repeat trial. On repeat trials the previous stimulus contrast is repeated. The side on which the stimulus is presented is not randomly selected but rather drawn from a normal distribution with a standard deviation of 0.5 and whose mean is the proportion of the previous 10 responses that were 'rightward' (that is, turning the wheel clockwise from the point of view of the mouse). If the sampled value is less than 0.5, the stimulus is presented on the left, otherwise on the right. This is a form of soft counter biasing where the more the mouse turns to one side, the more likely the stimulus will appear on that side (on repeat trials only).

The proportion of correct responses over the previous 50 trials for each contrast and trial side is recorded and used to update the contrast set: if the mouse performs at or above 70% for all 50% and 100% contrast 'left' trials and likewise for 'right' trials (excluding 'repeat' trials), the 25% contrast is added to the set. If the mouse performs over 70% at the 25% 'left' trials (and likewise for the 'right' trials), the 12.5% contrast is added to the set. 200 trials after this, the 6% contrast is introduced regardless of performance. Likewise the 0% is added and the 50% removed from the set 400 and 600 trials after the introduction of the 12% contrast, thus reducing the total number of high contrast trials. The performance and contrast set is carried over between sessions.

A session is ended for the following reasons:

1. The mouse fails do more than 400 trials in the first 45 minutes and fails to do more than 45 trials in the last 5 minutes;
2. The mouse has been training for > 45 minutes and in the last 5 minutes it does fewer than 45 trials
3. The mouse has been training for > 90 minutes.

The mouse is removed within five minutes of the session ending.

If the mouse completed over 200 trials in the previous session, the reward volume is lowered by 0.1ul until a volume of 1.5ul is reached. If the mouse has received less than its minimum required ($\sim 1\text{ml}/25\text{gr}$) during the previous session, the reward volume is increased by 0.1ul up to 3ul.

Training stage timeline

Outline

- Quarantine / acclimatisation period in case of transport.
- Perform a headbar implant surgery (see **Appendix 1: IBL protocol for headbar implant surgery in mice**) . This surgery can be done on any day of the week unless stated otherwise in institutional guidelines.
- The post-surgery recovery time is fixed to 7 days (counting the day of the surgery), with water *ad libitum*.
- Passed the post-surgery recovery time, the water restriction will start.
- Upon starting water restriction, mice will be handled under the habituation protocol.
- Following habituation, mice will undergo the training protocol.
- Once training starts, mice have to be trained for at least **3 days in a row**. If training starts on Wednesday/Thursday/Friday, researchers take upon themselves to come in during the weekend and train their animals.

Surgery and Water restriction timeline

- **day 1** : headbar implant surgery.
- **days 2-7** : post-surgery recovery (5-10 min handling; weight measurement).
- **day 8** : start water restriction (10 min handling; repeated weight measurements).

Note: On Day 8, in order to obtain a stable value for the baseline weight, measure the mouse's weight **several times in the day** while the mouse still has *ad libitum* access to water. The baseline weight will later be entered in the Alyx database to compute weight change.

Then, start water restriction (by removing the water bottle).

- **days 9-10(+)** : weight stabilisation and handling (10 min handling; water from syringe in hand or water in cage on weekends; weight measurement).

Note: The amount of days can be extended so as to start pre-habituation on a chosen day, hence the (+).

Each day while handling, give the minimum water amount (regular water, amount as required by institutional protocol). Do this for at least 7 days, or until the weight has stabilized. Give this water to the mouse placed in your hand from a syringe (preferably with a Gauge needle).

Habituation timeline

- **day 11** : 10 min free exploration of rig, water from syringe in hand.
- **day 12** : 20 min head-fixation with `habituationWorld` (wheel fixed¹); the mouse is brought out slightly so the paws are on the wheel.
- **day 13** : 40 min head-fixation with `habituationWorld` (wheel fixed).
- **day 14** : 60 min head-fixation with `habituationWorld` (wheel fixed).

¹ The wheel can be fixed by applying two strips of tape across the wheel, anchoring it to the mount. For extra security add a couple of blobs of Blu-Tac under the wheel.

Important: during `habituationWorld`, the animals will not drink their required amount. Supplement them with normal water (from a syringe, in your hand) at the end of the day.

Signs of stress during head-fixation

If you notice any of the signs below, remove the animal from head-fixation immediately.

1. Vocalisation.
2. Squinting eyes.
3. Mucous secretion from the eyes that the animal does not clean when headfixed. This disappears and gets cleaned once the animal is taken off head-fixating.
4. Excessive pooping/peeing.
5. An erect/moving tail which denotes they are uncomfortable with the head-fixed position.

Training timeline

- **day 17+** : `trainingChoiceWorld` task (wheel unlocked).
 - Upon starting mice have to be trained for at least **3 days in a row**.
 - Until the mouse reaches the criterion for learning “Level 1” (see section below for definition).
- **once “Level 1” is reached**: `biasedChoiceWorld` task.
 - Until the mouse reaches the criterion for learning “Level 2” (see section below for definition).
- **once “Level 2” is reached**: the animal is transferred onto another apparatus to perform electrophysiological recording.

Criterion

Criterion to assess learning

Level 1

The mouse is classified as having learned the task `trainingChoiceWorld` (Level 1) once three criteria have been met:

1. The 0% and 6% contrasts have been introduced.
2. In each of the last three consecutive sessions the mouse completed over 200 trials and performed over 80% on the easy (100% contrast) trials.
3. Using all trials from the last three sessions, a psychometric curve fit with four parameters: bias, lapse right, lapse left and threshold, must meet the following criteria: the absolute bias must be below 16, the threshold below 19 and each lapse below 0.2.

Not trainable

If the animal has done `trainingChoiceWorld` for over 40 days, but has not achieved the Level 1 trained status (i.e. has not moved on to `biasedChoiceWorld`), animal can be (but does not have to be) removed from pipeline.

Level 2

Once the mouse is classified as 'trained' in Level 1, it moves on to `biasedChoiceWorld`. Here, the repeat trials are removed. Each session starts with 90 trials, 10 of each contrast, with 50/50 prior probability.

After that, the probability of each trial being on the left strictly alternates between 0.8 and 0.2 every (the probability of the first biased block being 0.8 is a half). The number of trials for each block is drawn from an exponential distribution with a mean of 50, producing a flat hazard rate where the length is between 20 and 100 trials.

The mouse is classified as having learned the task `biasedChoiceWorld` (Level 2) once these criteria have been met:

- The mouse has to perform 3 consecutive sessions of at least 400 trials each, with above 90% correct on easy contrasts (collapsing across block types).
- The psychometric curves based on last 3 sessions (separately fit on each block type) should satisfy:

- Lapse rates measured on asymmetric blocks have to be below 0.1 (this comprises 4 values: lapse low and lapse high for both 80/20 and 20/80 blocks).
- The bias shift has to be bigger than 5 %.
 - The bias shift is defined as the bias in the 80/20 block minus the bias in the 20/80 block.
- The median reaction time across the 3 session at 0% has to be below 2 seconds.

Task parameters

Visual stimulus

Full contrast set: {1, 0.5, 0.25, 0.125, 0.06, 0}
Probability: {2/11, 2/11, 2/11, 2/11, 2/11, 1/11}
Starting contrasts: {1, 0.5}
Spatial frequency: 1/10 cyc/°
Sigma: $(7^\circ)^2$
Azimuth: $\pm 35^\circ$
Elevation: 0°
Orientation: 0°
Phase: randomized each trial
Threshold displacement: 35° , i.e. at azimuth $\pm 70^\circ$ (side) or 0° (center)

trials over which to calculate performance for each contrast: 50
trials required after 12.5% contrast before introducing 0% contrast: 200

Initial wheel gain: $8^\circ/\text{mm}$
Minimum # trials for gain change: 200
Low wheel gain: $4^\circ/\text{mm}$

Onset tone

Waveform: sinewave
Duration: 100 ms
Frequency: 5 kHz
Ramp duration: 10 ms
Phase: 2π rad
Sample rate: 192 kHz (Xonar sound card)

Negative feedback tone

Waveform: white noise
Duration: 0.5 seconds

Reward

Starting (maximum) reward size: 3ul
Decrease reward:
Minimum # trials in previous session for reward decrement: 200
Reward decrement: 0.1ul
Minimum reward: 1.5 uL for sugar water, 2 uL for normal water
Decrementing reward start on first session of trainingChoiceWorld

Increase reward:

If the mouse has received less than its minimum required (~1ml/25gr) during the previous session, the reward volume is increased by 0.1 ul up to (max) 3 ul.

Trial side proportions

Untrained trial side proportions: {0.5}
Repeat trial contrasts: {1, 0.5}
trials over which to calculate bias: 10
Repeat trial side proportions: $\sim N(\text{bias}, 4)$
Learned trial side proportions: {0.5, 0.8, 0.2} (last two strictly alternate)
Trial side block length, $1:10 + x$, where $x \sim \exp(50)$, $1 \in 20 \leq Z^+ \leq 100$

Timing

Pre-stimulus quiescence time, t : $0.2 + x$, where $x \sim \exp(0.35)$, $t \in 0.2 \leq R \leq 0.5$
Inter-trial interval (after stimulus offset): 0.5 seconds
Feedback period (stimulus on): if correct 1 second, otherwise 2 seconds
Response window: 60 seconds

References

- International Brain Laboratory et al. (2020) A standardized and reproducible method to measure decision-making in mice
- N. D. Gaubitch et al. (2011) [Bayesian Adaptive Method for Estimating Speech Intelligibility in Noise](#)
- The DataJoint code computing the psychometric curve is here: [int-brain-lab/IBL-pipeline/ibl_pipeline/analyses/analysis_utils.py](#)
- It uses this function to get the parameter fits: [int-brain-lab/analysis/python/psychofit.py](#)
- And uses those parameters here for defining the training criteria: [int-brain-lab/IBL-pipeline/ibl_pipeline/analyses/behavior.py](#)