



Appendix 2: IBL protocol for mice training

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The latest version of this protocol can be found [on Figshare via this specific link](#).

All IBL protocols cited in the article can be found [on Figshare via this master link](#).

Please cite the associated article when using any of these materials and protocols:

The International Brain Laboratory et al. (2020) *Standardized and reproducible measurement of decision-making in mice*. bioRxiv, 909838.
<https://doi.org/10.1101/2020.01.17.909838>



Appendix 2:

IBL protocol for mice training

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Introduction

Each mouse is trained on a 2-alternative forced-choice trial-based task requiring it to turn a wheel in order to move a visual stimulus (a Gabor patch) from the periphery to the center of its visual field (see Figure 1A in *International Brain Laboratory et al., 2019*).

Each mouse goes through three training protocols :

- `habituationWorld`¹ during which for each trial the Gabor patch appears in the periphery before instantaneously moving to the center of the screen after a delay, without interaction with the wheel. Shortly after the Gabor patch appears in the center of the screen, the mouse receives a water reward.
- `trainingChoiceWorld`² during which for each trial the Gabor patch has to be actively moved by the mouse via the wheel to the center of its visual field. The Gabor patch is presented on either the left or the right side of the screen with equal probability (50/50). There are three possible trial outcomes: a timeout, an incorrect response (resulting from the mouse turning the wheel too far in the incorrect direction), or a correct response (when the mouse successfully moves the Gabor patch to the center of the screen, resulting in a water reward). This is known as the “basic task”.
- `biasedChoiceWorld`³ during which for each trial the Gabor patch has to be actively moved by the mouse via the wheel. The probability that the Gabor patch is presented on either the left or the right side of the screen depends on the trial block. There are two types of blocks, which strictly alternate: in one block type, the Gabor patch is presented on the left and right with probabilities of 0.2 and 0.8 (20:80) respectively, and in the other block type the Gabor patch is presented on the left and right with probabilities of 0.8 and 0.2 (80:20) respectively. There are three possible trial outcomes, the same as in `trainingChoiceWorld`. This is known as the “full task”.

The Gabor patch may be presented at several contrasts (from 0% to 100%) depending on the protocol and task stage within a protocol. Psychometric curves can be computed for the three different types of trial blocks: 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*).

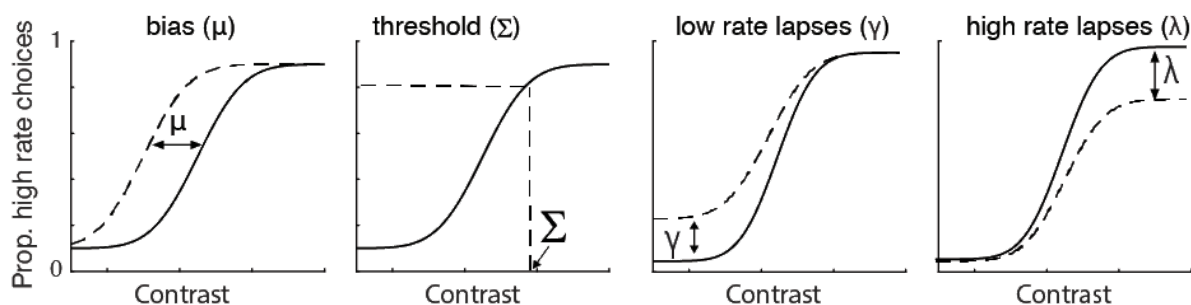
¹ Code: [tasks/ jblrig_tasks_habituationChoiceWorld](#)

² Code: [tasks/ jblrig_tasks_trainingChoiceWorld](#)

³ Code: [tasks/ jblrig_tasks_biasedChoiceWorld](#)



Psychometric curve metrics



The psychometric curve⁴ is determined by:

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

Where

γ is the low lapse rate

λ is the high lapse rate

xx is a vector of Gabor patch contrast values

μ is the bias (commonly referred to as the error function “threshold”)

Σ is the rate of change of performance (commonly referred to as the error function “slope”)

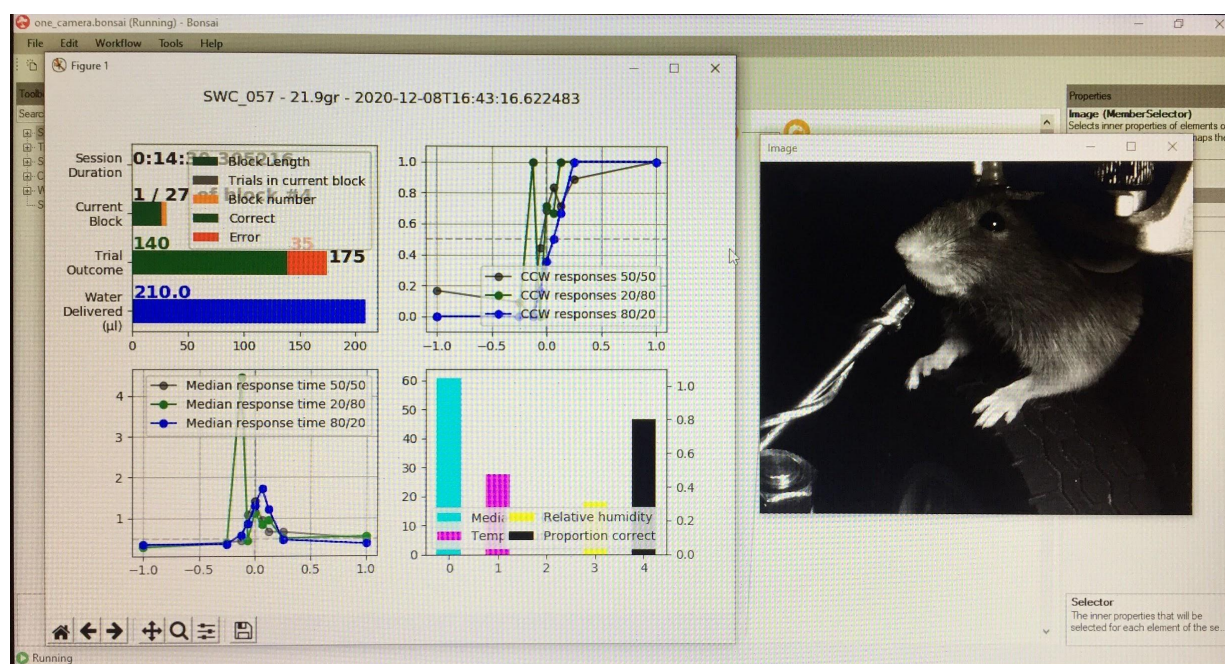
⁴ Code: [psychofit.py](https://github.com/IBL/psychofit.py); DataJoint pipeline code: [analysis_utils.py/compute_psych_pars](https://github.com/IBL/analysis_utils.py/compute_psych_pars)



User interface

The pyBpod interface is used to interact with the Bpod device. Documentation on pyBpod can be found here: <https://pybpod.readthedocs.io/en/latest/>

Specifically, the interface tailored for monitoring the animal during the task displays statistics on the session and animal performance (session duration, type of trial presented and outcome, water amount delivered; psychometric curves; reaction time curves; ambient measures such as humidity or temperature).



Placing the mouse on the setup

Headplate holder

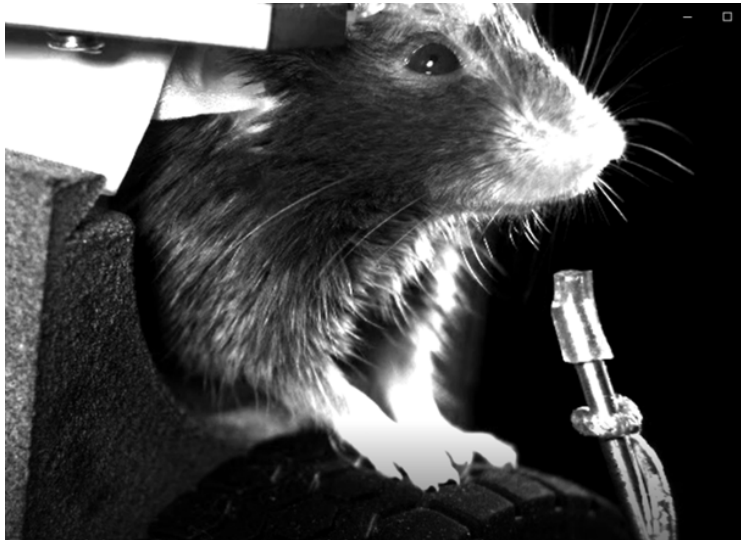


Figure: Well-adjusted position of a mouse in the IBL rig setup.

The headplate holder position should be adjusted (in the up-down and in backward-forward directions) so that the mouse's paws are centered on the wheel, and the mouse comfortably rests in the body holder. The mouse's forelimbs should be at a slight angle compared to the horizontal axis, as they would be during normal resting conditions (see example image below from a C57Bl6 mouse).



Figure: Resting position of a C57Bl6 mouse⁵.

Lick port

The lick port should be placed at a small distance from the mouse, so the mouse can easily reach water drops with its tongue. One should ensure both the tongue and the water drop are visible on the video recording.

⁵ Reference: The Jackson Laboratory - [017537 - C57BL/6J-Sting1](https://www.jax.org/Strain-Resource/017537-C57BL/6J-Sting1)

Note: at the beginning of the training phase, i.e. prior to the mouse being trained, the lick port can be placed slightly closer to the mouse's mouth so the mouse recognises more easily that there is water available.

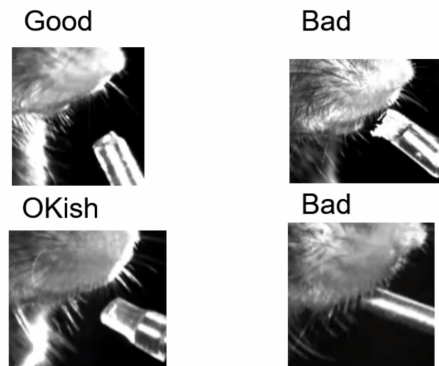


Figure: Well-adjusted (left column) or not (right column) lick port positioning.

Training stage description

Habituation CW

On the first two days, a mouse is 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 mouse is habituated in the rig for three days, where it is head-fixed with the wheel immovable. The mouse is head-fixed for 15-20 minutes on the first day, then 20-40 minutes the second day, and for 60 minutes the third day. The Gabor patch is presented at one of two random initial locations on each trial (+ or -35 degrees in the azimuth, 0 degrees elevation relative to the center of the visual field of the mouse) for an average of 10 seconds (drawn from a normal distribution with a standard deviation of 2 seconds). The Gabor patch then appears in the center of the screen, and 500 milliseconds later a 3 micro-liter reward is given. The Gabor patch stays in the center for another 500 milliseconds (so for 1 second in total) before it disappears. A period of grey screen follows before the next Gabor patch is presented. This grey screen period is on average 1 second.

The Gabor patch presented during this habituation is full-contrast, comprising a vertical sinusoidal grating with a spatial frequency of 1/10th of a cycle per visual degree masked by a Gaussian window of 7 square degrees⁶. The spatial phase of the Gabor patch is randomized each trial, drawn from a continuous uniform distribution.

⁶ Code: [_iblrig_tasks_habituationChoiceWorld/task_settings.py](#)

Training CW

On the sixth day (the fourth head-fixed day), the wheel is unlocked and the “active” phase of training begins. The contrast of the presented Gabor patch is now randomly chosen from a set of contrasts each trial, following a uniform distribution (except when the 0 contrast is introduced, see section Training phases below). Initially, this set comprises just two contrasts: 50% and 100%. The mouse must perform a series of trials where it moves the Gabor patch from the initial position to the center of the screen to obtain a water reward. The mouse moves the stimulus by turning the wheel in front of it. At the beginning of each trial, the mouse must not move the wheel for a fixed, “quiescent” period for the trial to continue. The duration of this period is between 400 and 700 milliseconds (computed as 200 ms + a duration drawn from an exponential distribution with a mean of 350 milliseconds, min and max of 200-500 milliseconds). If the wheel moves during this period, the timer is reset to zero, and again the wheel must remain motionless for the same period of time in order for the trial to continue. If the mouse successfully keeps the wheel “still” (within 2 degrees of wheel turn) during this quiescent period, a Gabor patch appears on either the left or right with equal probability, and an onset tone is played for 100 milliseconds. The tone consists of a 5 kilohertz sine wave with a 10 millisecond ramp duration to prevent onset artifacts⁷.

As soon as the Gabor patch appears, the mouse has 60 seconds to move it to the center of the screen. There are 3 possible outcomes⁸:

- If the mouse successfully moves the Gabor patch to the center of the screen, the trial is marked as a **correct** response, and the mouse receives a 3 microliter reward.
- If the mouse incorrectly moves the Gabor patch too far in the opposition direction, i.e. 35 degrees further to the periphery, the trial is marked as an **incorrect** response.
- If neither threshold is reached within 60 seconds, the trial is marked as a **time-out**. After both the incorrect and time-out outcome, the Gabor patch is fixed at 70 degrees azimuth for 2 seconds on the side the stimulus originated on, and a noise burst is played for half a second, after which the next trial begins.

At the onset of this active phase of training the stimulus moves 8 visual degrees per millimetre of movement at the wheel surface. If the mouse completes at least 200 response trials within a session, the gain of the wheel for the following session is halved, to 4 visual degrees per millimetre⁹.

Debiasing

Repeat trials occur if the response was incorrect and the Gabor patch contrast was **easy** ($\geq 50\%$). On repeat trials the previous contrast is repeated and the side on which the Gabor patch is presented is not randomly selected, but rather drawn from a normal distribution with a

⁷ Code: [sound.py/make_sound](#)

⁸ Code: [iblrig_tasks_trainingChoiceWorld.py](#)

⁹ Code: [adaptive.py/init_stim_gain](#)

standard deviation of 0.5 and mean of the fraction of the previous 10 responses that were 'rightward' (that is, when the wheel was turned clockwise from the point of view of the mouse)¹⁰.

For example, if the last 10 trials were:

L-R-R-L-R-L-L-R-R-L, where R means 'rightward' responses, the $\text{fraction}(\text{R}) = 5/10 = 0.5$

If the sampled value is strictly less than 0.5, the Gabor patch is presented on the left, otherwise on the right. This is a form of soft counter biasing where the more the mouse turns in one direction, the more likely it is that this will be incorrect on the next trial, meaning the correct movement would be to the opposite side (again, on repeat trials only). See '*Trial side proportions*' in the '*Task parameters*' section below for exact implementation details.

¹⁰ Code: [trial_params.py/_next_position](#)

Training phases

The active phase of training comprises 6 stages. At each new stage, a contrast value is either added or removed from the set¹¹. The performance and contrast set is carried over between sessions. Below each stage is summarized.

1. Only 50% and 100% contrasts are presented. The proportion of correct responses over the previous 50 trials is recorded. To progress, the mouse must perform at or above 80% correct for each contrast on both sides (excluding “repeat” trials).
2. The 25% contrast is added to the set. As above, to progress the mouse must perform at or above 80% on each contrast.
3. The 12.5% contrast is added to the set. To progress the mouse must perform 200 trials, regardless of performance.
4. The 6% contrast is added to the set. To progress the mouse must perform 200 trials, regardless of performance.
5. The 0% contrast is added to the set. The side (and thus correct movement) for the 0% contrast trials is chosen randomly between the right and left side with equal probability. To progress the mouse must perform 200 trials, regardless of performance.
6. The 50% contrast is removed from the set, resulting in a final contrast set of [100, 25, 12.5, 6, 0], and repeat trials are dropped.

The probability to choose a contrast value is kept uniform across the set, except when the 0% contrast is introduced. For example, in a session with a contrast set being [100, 50, 25], there is $\frac{1}{3}$ chance to draw a given contrast on a trial. This rule is valid for the training phases 1-4 above. However, when the 0% contrast is present in the set (training phases 5-6), the probability of drawing the 0% contrast is half compared to others. For example in phase 6: the probability of drawing the [100 : 25 : 12.5 : 6 : 0] % contrast is [2 : 2 : 2 : 2 : 1]/9.

End of session criteria

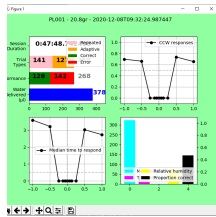
A session may be ended for the following reasons¹²:

1. **Session too long** [red] : The mouse has been training for > 90 minutes
2. **Not enough trials** [green] : The mouse fails to do more than 400 trials in the first 45 minutes
3. **Slow-down** [yellow]: The mouse completes over 400 trials in the session and the median response time (defined as the time from stimulus on to threshold) over the past 20 trials is over five times longer than the median response times over the entire session.

Note: The pyBpod GUI automatically indicates whether one of these criteria is reached by changing the background color of the main plot, but the task keeps on running. It is up to the experimenter to end the task once such an indication is given.

¹¹ Code: [trial_params.py/ update_contrast_set](#)

¹² Code: [misc.py/check_stop_criteria](#)



Example GUI display where the “Not enough trials” [green] criteria is reached

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

Reward volume

If the mouse completed over 200 trials in the previous session, the reward volume is automatically lowered by 0.1 microliters for the next session, but cannot go lower than a floor of 1.5 microliters. If the mouse received less than its minimum required daily dose (~1 milliliter/25 grams of body weight) during the previous session, the reward volume is increased by 0.1 microliters for the next session, but cannot go above a ceiling of 3 microliters.¹³

Biased CW

Here, the repeat trials are removed, and will no longer be present in any further tasks.

Each session starts with 90 trials, where the probability of a Gabor patch to appear on the left or right side is 50%¹⁴. Specifically, the 100%, 25%, 12.5%, and 5% contrast trials are each presented 10 times on each side, and the 0% contrast is presented 10 times in total (i.e. the ratio of the [100 : 25 : 12.5 : 6 : 0] % contrasts is set at [2 : 2 : 2 : 2 : 1]).¹⁵ The side (and thus correct movement) for the 0% contrast trials is chosen randomly between the right and left with equal probability. This initial block of 90 trials is called the unbiased block (50/50).

After the unbiased block, trials are presented in two different (biased) block types: in one block, the Gabor patch is presented on the left and right with probabilities of 0.2 and 0.8 (20:80) respectively, and in the other block type the Gabor patch is presented on the left and right with probabilities of 0.8 and 0.2 (80:20) respectively. In biased blocks, the ratio of the contrasts differs from above, whereby the ratio of the [100 : 25 : 12.5 : 6 : 0] % contrasts is set at [1 : 1 : 1 : 1 : 1]. In a given session, there is an equal probability of the first biased block to be set as 20:80 or 80:20. Block types strictly alternate over a given session.¹⁶

¹³ Code: [adaptive.py/init_reward_amount](#)

¹⁴ Code: [blocks.py/init_block_len](#)

¹⁵ Code: [misc.py/get_biased_probs](#)

¹⁶ Code: [blocks.py/calc_probability_left](#)

For example, in a given session, the probability (%) of the Gabor patch being presented on the left/right in the first 5 blocks would be: (50:50) - (20:80) - (80:20) - (20:80) - (80:20) .

The number of trials for each biased block is drawn from an exponential distribution with a mean of 60, with a flat hazard rate where the minimum and maximum number of trials per block is 20 and 100, respectively.¹⁷

Ephys CW

This is the task that was used during acquisition of neural data.

Each session starts with 90 trials, where the probability of a Gabor patch to appear on the left or right side is 50%¹⁸. Specifically, the 100%, 25%, 12.5%, and 5% contrast trials are each presented 10 times on each side, and the 0% contrast is presented 10 times in total (i.e. the ratio of the [100 : 25 : 12.5 : 6 : 0] % contrasts is set at [2 : 2 : 2 : 2 : 1]).¹⁹ The side (and thus correct movement) for the 0% contrast trials is chosen randomly between the right and left with equal probability. This initial block of 90 trials is called the unbiased block (50/50).

After the unbiased block, trials are presented in two different (biased) block types: in one block, the Gabor patch is presented on the left and right with probabilities of 0.2 and 0.8 (20:80) respectively, and in the other block type the Gabor patch is presented on the left and right with probabilities of 0.8 and 0.2 (80:20) respectively. The ratio of the contrasts remains as above ([2 : 2 : 2 : 2 : 1]). In a given session, there is an equal probability of the first biased block to be set as 20:80 or 80:20. Block types strictly alternate over a given session.²⁰

For example, in a given session, the probability (%) of the Gabor patch being presented on the left/right in the first 5 blocks would be: (50:50) - (20:80) - (80:20) - (20:80) - (80:20) .

The number of trials for each biased block is drawn from an exponential distribution with a mean of 60, with a flat hazard rate where the minimum and maximum number of trials per block is 20 and 100, respectively.²¹

In order for sessions to be comparable across animals, trial sequences were pre-generated for 12 sessions²², and selected upon session start. 12 sessions were generated in order to avoid animals seeing multiple times the same trial sequence over consecutive sessions. Typically, the sequence #1 is selected upon the first ephys session of a subject, and so on.

¹⁷ Code: [blocks.py/get_block_len](#), [misc.py/texp](#), [task_settings.py](#)

¹⁸ Code: [blocks.py/init_block_len](#)

¹⁹ Code: [misc.py/get_biased_probs](#)

²⁰ Code: [blocks.py/calc_probability_left](#)

²¹ Code: [blocks.py/get_block_len](#), [misc.py/texp](#), [task_settings.py](#)

²² Code: https://github.com/int-brain-lab/iblr/tree/master/tasks/_iblr_tasks_ephysChoiceWorld/sessions

Criteria to assess learning

See the implementation of these criteria on DataJoint [here](#).

Trained

The mouse is classified as having learned the `trainingChoiceWorld` protocol (also known as the “basic task”) after meeting performance criteria in two stages:

Trained 1a:²³

1. The 0% contrast has been introduced (stage 5).
2. In each of the last three consecutive sessions, the mouse completed over 200 trials and performed over 80% on the easy (contrast $\geq 50\%$) 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.

Trained 1b:²⁴

1. In each of the last three consecutive sessions, the mouse completed over 400 trials and performed over 90% on the easy (contrast $\geq 50\%$) trials.
2. 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 10, the threshold below 20 and each lapse below 0.1.
3. The median reaction time across the 3 sessions for the 0% contrast trials is below 2 seconds.

NB: The criteria for level 1b are stricter than those for 1a, however it is possible for a mouse to go straight to level 1b.

Untrainable

If the mouse has been run on `trainingChoiceWorld` for over 40 days, but has not achieved the “trained_1b” status (i.e. has not moved on to `biasedChoiceWorld`), the mouse may be (but does not have to be) removed from the pipeline.

Unbiasable

²³ Code: https://github.com/int-brain-lab/IBL-pipeline/blob/master/ibl_pipeline/analyses/behavior.py#L737

²⁴ Code: https://github.com/int-brain-lab/IBL-pipeline/blob/master/ibl_pipeline/analyses/behavior.py#L686

If the mouse has been on `biasedChoiceWorld` for over 40 days, but has not achieved “ready4ephysrig” status, the mouse may (but does not have to be) removed from the pipeline.

Ready for ephys rig

Once the mouse is classified as “trained_1b”, it moves on to `biasedChoiceWorld`.

Addendum 2021-11-01_: If the mouse has reached “trained_1a”, but not “trained_1b”, it can also be moved to `biasedChoiceWorld` on the 5th session after reaching “trained_1a”. For example, a mouse reaches “trained_1a” on Wednesday 2nd Oct, and is trained on Thu-Fri-Mon-Tues without reaching “trained_1b”. On Wednesday 9th Oct, it can be moved to `biasedChoiceWorld`.

The mouse is classified as having learned the `biasedChoiceWorld` protocol (“ready4ephysrig”) once three criteria have been met²⁵:

1. In each of the last three sessions the mouse completed at least 400 trials, and performed over 90% on the easy contrasts ($\geq 50\%$), regardless of the block type
2. The psychometric curves based on the last 3 sessions (separately fit for each block type) satisfy the following criteria:
 - a. Lapse rates measured on asymmetric blocks are below 0.1 (this comprises 4 values: lapse low and lapse high for both 80:20 and 20:80 blocks).
 - b. The bias shift has to be $> 5\%$.
 - i. The bias shift is defined as the bias in the 80:20 block minus the bias in the 20:80 block.
3. The median reaction time across the 3 sessions for the 0% contrast trials is below 2 seconds.

²⁵ Code:

https://github.com/int-brain-lab/IBL-pipeline/blob/master/ibl_pipeline/analyses/behavior.py#L606



Task parameters

Notation:

$\exp(\mu)$: Exponential distribution (mean)
 $N(\mu, \sigma)$: Normal distribution (mean, standard deviation)
R: Real number
 Z^+ : Positive integer number

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: $\sim U\{0, \pi\}$
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: $\sim N(\text{bias}, 0.5)$

Learned trial side proportions: {0.5, 0.8, 0.2} (last two strictly alternate)

Trial side block length, x is drawn from a geometric distribution of mean 51 where $x \in 20 \leq Z^+ \leq 100$.

Timing

Pre-stimulus quiescence time, t : $0.2 + x$, where $x \sim \exp(0.35)$, $x \in 0.2 \leq R \leq 0.5$, $t \in 0.4 \leq R \leq 0.7$

Inter-trial interval (after stimulus offset): 0.5 seconds

Feedback period (stimulus on): if correct 1 second, otherwise 2 seconds

Response window: 60 seconds

Training stage timeline

Outline

- Quarantine / acclimatization 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*.
- After the post-surgery recovery time, the water restriction will start.
- Upon starting water restriction, each mouse will be handled under the habituation protocol.
- Following habituation, each mouse will undergo the training protocol.
- Once training starts, each mouse must be trained for at least **3 days in a row**. If training starts on or after a Wednesday, researchers take upon themselves to come in during the weekend to perform animal training.

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 stabilization 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-habitation on a chosen day, hence the (+).

Each day while handling, give the minimum water amount (regular water, minimum amount as required by institutional protocol). This should be done for at least 7 days, or until the weight has stabilized. The water should be administered to the mouse from a syringe (preferably with a plastic syringe needle) by hand.



Habituation timeline

- **day 11** : 10 min free exploration of rig, water from syringe in hand.
- **day 12** : 20 min head-fixation with `habituationWorld`²⁶ (wheel locked²⁷); the mouse is brought out slightly so the paws are on the wheel.
- **day 13** : 40 min head-fixation with `habituationWorld` (wheel locked).
- **day 14** : 60 min head-fixation with `habituationWorld` (wheel locked).

Important: during `habituationWorld`, the mouse will not drink its required amount. The mouse should be supplemented with normal water (from a syringe, by hand) at the end of the day.

Signs of stress during head-fixation

If any of the signs below are observed, remove the mouse from head-fixation immediately.

1. Vocalization.
2. Squinting eyes.
3. Mucous secretion from the eyes that the mouse does not clean when head-fixed. This disappears and gets cleaned once the mouse is taken off the head-restraint.
4. Excessive defecation/urination.
5. A persistently erect/moving tail, which indicates the mouse is uncomfortable with the head-fixed position.

See below for signs of discomfort in freely-moving mice.

²⁶ Code: [_iblrig_tasks_habituationChoiceWorld.py](#)

²⁷ The wheel can be locked in place by applying two strips of tape across the wheel, anchoring it to the mount. For extra security, a couple of blobs of Blu-Tac can be added under the wheel.



The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in awake animals. Each animal should be observed for a short period of time to avoid scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening - Closing of the eyelid (narrowing of orbital area) - A wrinkle may be visible around the eye			
Nose bulge - Bulging on the bridge of the nose - Vertical wrinkles on the side of the nose			
Cheek bulge Bulging of the cheeks			
Ear position - Ears rotate outwards and/or backwards, away from the face - Ears may fold to form a 'pointed' shape - Space between the ears increases			
Whisker change - Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' - Whiskers may clump together - Whiskers lose their natural 'downward' curve			

Training timeline

- **day 15+ :** trainingChoiceWorld task (wheel unlocked).
 - Upon starting, each mouse must be trained for at least **3 days in a row**.
 - Until the mouse reaches the criteria for having learned (see section below for definition).
- **once "trained 1b" status is reached:** biasedChoiceWorld task.

- Until the mouse reaches the criteria for having learned (see section below for definition).
- **once “ready for ephys rig” status is reached:** the mouse is transferred onto another apparatus to perform electrophysiological recording.



Water restriction

Weight management

Depending on institutional licences, mice have to stay either above the threshold of 85% of their baseline weight (on free water), or above 85% of their age-adjusted baseline weight.

Guidelines for weight recovery

If the mouse weight drops below **87%** ,

- Alyx presents the user a *gentle* warning.
- Guideline of immediate action:
 1. give fix amount of water (0.5mL water / 25g mouse),
 2. *or* place in recovery cage (free water) for fixed amount of time (1h),
 3. *or* give special food (peanuts and raisins),
 4. *or* do nothing

If the mouse weight drops below **85%** ,

- Alyx presents the user a *real* warning
- Guideline of immediate action:
 - Same as for 87% (apply step 1-3, either), monitor the weight every hour
 - Place mouse in water restricted cage only when animal weight reaches 87%, otherwise repeat steps 1-3 (either)
 - If the weight has not sufficiently increased by the time the experimenter goes home, leave the animal in the recovery cage overnight.

The recovery cage contains: water (ad lib), hydrogel, **diet gel**, wet food, and snacks.

Water on weekdays (Mon-Fri)

- In the rig, mice drink sucrose water (10% concentration: 10g/100mL).
- Top-up at the end of the session (if needed) is with **hydrogel**, to reach minimum volume of daily water required by the local protocol. Wait **at least an hour** after the task before administering top-up.

Water on weekend days (Sat-Sun)

Two possibilities:

1. ad lib water with citric acid at 2% (Reinagel, Front Behav Neurosci 2018).
2. measured water delivered to individual mice to reach minimum volume of daily water required by the local protocol.



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](#)