TITLE: Progressive Ratio (PR) and Post-PR Extinction Learning (Post-PR EXT)

SOP NO.: mPR-v1

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1. INTRODUCTION

The progressive ratio (PR) test has been designed to measure the effort a rodent is willing to expend to receive a single reward, or motivation. Previous use of this test on touchscreens in both rodents and humans have been shown to be sensitive to differences in dopaminergic activity, suggesting excellent translational validity of this test for measuring motivation in rodents. The test is performed in specially designed touchscreen-based automated chambers, where a stimulus is repeatedly presented in a centre window with each trial. Mice are required to repeatedly respond to the stimulus until the ratio requirement is achieved, after which a reward is delivered. PR schedules require an increased number of responses be made with each trial following a linear ramp (e.g., +4 responses on every subsequent trial), thereby increasing the amount of effort required for a single reward with every trial. An extinction learning paradigm can also be run to assess changes in responses to reward withdrawal and related neural functioning (e.g., executive function).

2. EQUIPMENT

- Mouse Touch Screen Systems and ABET II http://lafayetteneuroscience.com/listing/mice-touch-chambers-components/
- Progressive Ratio (PR) Task with Cambridge Amendment from the Cambridge University Group, a file run within ABET II during training and evaluation

3. PROCEDURE

1.1. General Equipment:

- Best practice to test the hardware prior to every training or testing day. Ensure that the expected inputs and outputs are observed.
- All programs are found in PR subdirectory in the ABETII software[1].

[1] Pre-training stages (Stages 1-3) were adapted from pairwise discrimination schedules, and FR training (Stages 4-6) and high demand schedules (Stages 8 and 9) were modified versions of the standard PR schedule. As such, file names may differ.

- A quick test of the feeder should be done prior to every training or testing day. Manually switch on the feeder pump and make sure the food is delivered and remove clog if necessary[2].
- Make sure the PR mask is inserted (5 windows).
- Reward provided is Neilson Strawberry milkshake (Saputo Inc. Montreal Quebec. H1P1X8). This milkshake can be found in most grocery stores (including Loblaws and Superstore).

1.2. Pre-training

- Make sure your mice are food restricted to 85-90% of their free-feeding weight prior to the start.
- Provide strawberry milkshake to the mice in their home cages for at least 3 days immediately prior to training.
- Divide each group of subjects into counter-balanced sub groups containing both control and test mice to control for the time of day the experiment is performed, and the particular cabinet being used in case of an equipment failure.

1.3. Training Procedures

3.3.1 Basic training schedule

Generally, mice are given 1 session per day.

Stage 1a: Habituation 1

ABETII program file: PR Mouse Habituation 1
Duration: 1 session, 600 s (10 minutes)
Trial number: Unlimited
Description: This stage is designed to habituate the mouse to the touchscreen chamber itself. The mouse is left in the chamber for 10 mins. All lights are turned off. No stimulus or reward is presented. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.
Criterion: None

[2] Best practice is to also check that milkshake is still flowing between each animal being run in the touchscreen chamber.

Stage 1b: Habituation 2a

ABETII program file: PR Mouse Habituation 2 v2
Duration: 2 sessions, 1200 s (20 minutes)
Trial number: Unlimited
Description: This stage habituates the mouse to the reward collection process, including the reward magazine/food tray itself and secondary reward cues (e.g., tone and magazine light). The tray light is initially turned on. A tone is played and the food-tray/magazine is primed with strawberry milkshake delivered for 6000 ms (~150 µl). The program waits for the mouse to enter the food tray. When the mouse leaves the reward tray, the reward tray light is turned off. There is a 10-s delay before the tray light is turned on, a tone is played, and milkshake is then delivered for 800 ms (~20 µl)[3]. If the mouse is in the reward tray at the end of the 10-s delay, an extra 1 s is added to the delay. The procedure is repeated until the session ends. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.
Criterion: Drinks milkshake (none observed in tray).[4]

Stage 1b: Habituation 2b

ABETII program file: PR Mouse Habituation 2 v2
Duration: 1 session, 2400 s (40 minutes)
Trial number: Unlimited
Description: The mouse is left in the chamber for 40 min. Reward presentation is the same as described in Stage 1b. It is critical that the mouse is removed from the cabinet as soon as the habituation is complete.
Criterion: No milkshake found in tray at end of session.

Stage 2: "Initial touch"

ABETII program file: PR Mouse Initial Touch Training v2 - Short ITI
Duration: Minimum 1 session, 3600 s (60 minutes)
Trial number: 30
Description: This schedule trains the mouse to touch the stimulus and make the initial association of the stimulus with reward. Make sure that "Image Time" is 30 s, "Feed Pulse Time" is 800 ms, and ITI period is 4.5 s. The stimulus (white box[5]; same as is used in all following stages/schedules) is displayed in the centre window of the 5-window mask. All other windows are blank. After a delay (Image Time – 30 s), the stimulus is removed and

[3] Note that ABETII has a pre-set standard of 280ms (7µl) of strawberry milkshake delivered. The TCN Lab while at Cambridge increased this volume. The rationale is that animals that get few rewards on challenging tasks may remain more motivated as the reward is larger when it is delivered.
[4] If your mouse does not drink milkshake, you may wish to give the milkshake in the home cage with their food, check the weight of the animal, and give extra sessions.
[5] "Lamp" from the 5-Choice Serial Reaction Time (5-CSRT) task.

a reinforcer is delivered (Feed Pulse Time – 800 ms). Food delivery is accompanied by illumination of the tray light and a tone. The tone frequency is 3 KHz and the tone duration is 1000 ms. Entry to collect the food turns off the tray light and initiates the ITI. After the ITI period (4.5 s), the next trial begins and the stimulus is presented. However, if the mouse touches the stimulus within 30 s, the stimulus is removed upon response and the magazine light illuminates, the tone is played, and 3x the standard reward volume is dispensed (2400 ms). Collection of this reward again initiates the ITI and then progresses to the next trial. Training is performed with the house light off.
Criterion: Completion of 30 correct trials within 60 min, where correct is defined as trials in which the stimulus was touched within 30 s. Repeat sessions until criterion is achieved.

Stage 3: "Must touch"

ABETII program file: PR Mouse Must Touch Training v2
Duration: Minimum 1 session, 3600 s (60 minutes)
Trial number: 30
Description: This schedule trains the mouse that the stimulus must be touched to receive reward. Make sure **"**Feed Pulse Time" is set to 800 ms and ITI period is set to 4.5 s. The stimulus is presented in the centre window, with the others remaining blank. The stimulus remains on-screen until a response is made, after which the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. All other aspects of the task are the same as in Stage 2 (Initial Touch).
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

3.3.2. PR-specific training schedule[6]

Stage 4: "FR-1 training"

ABETII program file: FR 1 Training
Duration: Minimum 1 session, 3600 s (60 minutes)
Trial number: 30
Description: Make sure **"**Feed Pulse Time" is set to 800 ms and "Reward New Trial Delay" is set to 4.5 s. The stimulus is presented in the centre window, with the others remaining blank. The stimulus remains on-screen until a response is made, after which the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the

[6] It is best practice that procedures followed from this point on occur over consecutive days; i.e., no breaks in testing from first day of FR-1 training through to the final day of PR testing (which, depending on experimenter preference and/or purpose of study, may be the third day of the second block of PR-4 during basic PR-4 (subsection 3.4), the third day (PR-12) of the second block of high demand PR (subsection 3.5), or the third day of uncapped FR-5 (subsection 3.6).

stimulus reappearing on the screen. Only 1 response is required to receive reward. Stage 4, in that case, is essentially the same as Stage 3 (Must Touch).
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

Stage 5: "FR-2 training"

ABETII program file: FR 2 Training
Duration: Minimum 1 session, 3600 s (60 minutes)
Trial number: 30
Description: Make sure **"**Feed Pulse Time" is set to 800 ms, "Reward New Trial Delay" is set to 4.5 s, and ITI period is set to 0.5 s. The stimulus is presented in the centre window, with the others remaining blank. FR-2 requires 2 responses to be made before a reward is delivered. After the first response to the stimulus, the stimulus is briefly removed (500 ms, or 0.5 s), accompanied by a click, before being re-presented. The brief removal and the click-like tone are meant to signal to the mouse that an additional response is required. Once the ratio requirement is fulfilled for a trial (in this case, 2 responses), the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the stimulus reappearing on the screen.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

Stage 6: "FR-3 training"

ABETII program file: FR 3 Training
Duration: Minimum 1 session, 3600 s (60 minutes)
Trial number: 30
Description: Make sure **"**Feed Pulse Time" is set to 800 ms, "Reward New Trial Delay" is set to 4.5 s, and ITI period is set to 0.5 s. The stimulus is presented in the centre window, with the others remaining blank. FR-3 requires 3 responses to be made before a reward is delivered. After the first and second responses to the stimulus, the stimulus is briefly removed (500 ms, or 0.5 s), accompanied by a click, before being re-presented. Once the ratio requirement is fulfilled for a trial (in this case, 3 responses), the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the stimulus reappearing on the screen.
Criterion: Completion of 30 trials within 60 min. Repeat sessions until criterion is achieved.

Stage 6: "FR-5 training"

ABETII program file: FR 5 Training
Duration: 3 sessions, 3600 s (60 minutes)

Trial number: 30
Description: Make sure **"**Feed Pulse Time" is set to 800 ms, "Reward New Trial Delay" is set to 4.5 s, and ITI period is set to 0.5 s. The stimulus is presented in the centre window, with the others remaining blank. FR-5 requires 5 responses to be made before a reward is delivered. After the first four responses, the stimulus is briefly removed (500 ms, or 0.5 s), accompanied by a click, before being re-presented. Once the ratio requirement is fulfilled for a trial (in this case, 5 responses), the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the stimulus reappearing on the screen.
Criterion: Completion of 30 trials within 60 min on the third day/session (minimum). Repeat sessions until criterion is achieved.

3.4.Basic PR-4 schedule

The schedule is set up in blocks, with each block consisting of 3 consecutive days. There are 3 blocks, with two blocks of PR-4 separated by a single block of FR-5. PR-4 blocks are run as described below, whereas the FR-5 block is run exactly as during the PR-specific training (above, Stage 6). Though there is no explicit criterion for the FR-5 block, it is expected that the mice complete all 30 trials within 60 min given the prior PR-specific training paradigm. The FR-5 block is included to maintain and reinforce repeated responding to the stimulus. The PR-4 schedule requires the mouse to respond to the stimulus 4 times more than the previous trial, following a linear ramp. For instance, trial 1 requires 1 response, trial 2 requires 5 responses, trial 3 requires 9 responses, and so on.

Stage 7: "PR-4"

ABETII program file: PROGRESSIVE RATIO - PR 4
Duration: 6 sessions (minimum), 3600 s (60 minutes)
Trial number: Unlimited
Description: Make sure **"**Feed Pulse Time" is set to 800 ms, "Reward New Trial Delay" is set to 4.5 s, and ITI period is set to 0.5 s. The stimulus is presented in the centre window, with the others remaining blank. When multiple responses are required, the stimulus is briefly removed (500 ms, or 0.5 s), accompanied by a click, before being re-presented. Once the ratio requirement is fulfilled for a trial, the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the stimulus reappearing on the screen. The session ends either when 60 min has surpassed or after 5 consecutive min of inactivity.
Criterion: None.

3.5. High demand PR schedule (optional)

As with basic PR-4 (above), high demand PR is set up in blocks, with each block consisting of 3 consecutive days. There are 3 blocks total, with two blocks of high demand PR separated by a single block of FR-5. During the high demand blocks, PR schedules of increasing difficulty are used on each day, such as PR-4, PR-8, and PR-12 (details of PR-4 are listed above and those for PR-8 and -12 are listed below). As above, the FR-5 block is run exactly as during the PR-specific training (above; Stage 6). Though there is no explicit criterion for the FR-5 block, it is expected that the mice complete all 30 trials within 60 min given the prior PR-specific training paradigm. The FR-5 block is included to maintain and reinforce repeated responding to the stimulus.

Stage 8: "PR-8"

ABETII program file: PROGRESSIVE RATIO - PR 8
Duration: 2 sessions, 3600 s (60 minutes)
Trial number: Unlimited
Description: Make sure **"**Feed Pulse Time" is set to 800 ms, "Reward New Trial Delay" is set to 4.5 s, and ITI period is set to 0.5 s. The stimulus is presented in the centre window, with the others remaining blank. When multiple responses are required, the stimulus is briefly removed (500 ms, or 0.5 s), accompanied by a click, before being re-presented. Once the ratio requirement is fulfilled for a trial, the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the stimulus reappearing on the screen. The session ends either when 60 min has surpassed or after 5 consecutive min of inactivity.
Criterion: None.

Stage 9: "PR-12"

ABETII program file: PROGRESSIVE RATIO - PR 12
Duration: 2 sessions, 3600 s (60 minutes)
Trial number: Unlimited
Description: Make sure **"**Feed Pulse Time" is set to 800 ms, "Reward New Trial Delay" is set to 4.5 s, and ITI period is set to 0.5 s. The stimulus is presented in the centre window, with the others remaining blank. When multiple responses are required, the stimulus is briefly removed (500 ms, or 0.5 s), accompanied by a click, before being re-presented. Once the ratio requirement is fulfilled for a trial, the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the stimulus reappearing on the screen. The session ends either when 60 min has surpassed or after 5 consecutive min of inactivity.
Criterion: None.

3.6. Uncapped FR-5 (optional)

The uncapped FR-5 schedule consists of 3 consecutive days of FR-5, with unlimited trials. Uncapped FR-5 can serve as a control to assess whether altered responding on PR tasks were driven by effects on satiation.

Stage 10: "Uncapped FR-5"

ABETII program file: FR 5 Training
Duration: 3 sessions, 3600 s (60 minutes)
Trial number: Unlimited
Description: Make sure **"**Feed Pulse Time" is set to 800 ms, "Reward New Trial Delay" is set to 4.5 s, and ITI period is set to 0.5 s, and maximum trials set to unlimited. The stimulus is presented in the centre window, with the others remaining blank. After the first 4 responses of a trial, the stimulus is briefly removed (500 ms, or 0.5 s), accompanied by a click, before being re-presented. Once the ratio requirement is fulfilled for a trial (i.e., 5 responses), the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the new trial delay begins, after which the next trial starts with the stimulus reappearing on the screen. The session ends only after 60 min has surpassed.
Criterion: None.

3.7. Post-PR extinction learning schedule[7]

3.7.1. Re-Baseline to respond on an FR-1 schedule

Stage 11: "FR-1 re-baseline"

ABETII program file: Mouse Extinction pt 1 v2 - Baseline
Duration: Minimum 3 sessions, 3600 s (60 minutes)
Trial number: 30
Description: Make sure **"**Feed Pulse Time" is set to 800 ms and ITI period is set to 5 s. The stimulus is presented in the centre window, with the others remaining blank. The stimulus remains on-screen until a response is made, after which the stimulus is removed and reward is delivered (800 ms) with magazine light and tone. Upon reward collection, the ITI begins, after which the next trial starts with the stimulus reappearing on screen. Only 1 response is required to receive reward.
Criterion: Stabilized performance, defined as 3 consecutive days of no significant differences in time to complete all 30 trials.

Stage 12: "Extinction learning"

[7] Modified from standard Extinction Learning (EXT) schedules to be consistent with PR.

ABETII program file: Mouse Extinction pt 2 v2 - NO REWARD
Duration: Minimum 10 sessions, 3600 s (60 minutes)
Trial number: 30
Description: Ensure "Image Time" is set to 10 s and the ITI is set to 10 s. The stimulus is presented in the centre window, with the others remaining blank. The stimulus remains on-screen for 10 s or until a response is made, after which the stimulus is removed. No reward is delivered for responding to the stimulus. Once the stimulus is removed from the screen, the ITI begins and, once elapsed, the next trial starts.
Criterion: Minimum 10 sessions[8], after which the endpoint is designated as 2 consecutive days of at least 23 omissions made (i.e., 23/30 trials in which the stimulus was not touched).[9]

Additional Considerations

If the experimenter desires to change the salience/strength of reward used to compare within and/or across PR studies, the strawberry milkshake reward can be diluted with tap water to achieve a particular concentration (e.g., 50%, 20%, etc.).

If using high demand PR, other ratio schedules may be used so long as the demand increase is a multiple of the first ran with each day (i.e., in the high demand schedule listed in this SOP, 4, 8, and 12 are all multiples of 4). For instance, one could use PR-5, PR-10, and PR-15. By shifting the demand of each day, the experimenter can either make the schedule more or less challenging.

If using models of neurodegeneration, such as Alzheimer's transgenic mouse models, it may be preferred to use a truncated schedule, especially if the experimenter wants to use all 3 stages of the entire PR schedule (i.e., basic PR-4, high demand PR, and uncapped FR-5). The entirety of the PR schedule may be too long considering such rodent models have an increasing degree of neurodegeneration with time, especially if the mice are tested at later ages*. A possible truncated schedule will be made available shortly.*

Recommended Readings

Heath, C. J., Bussey, T. J., & Saksida, L. M. (2015). Motivational assessment of mice using the touchscreen operant testing system: Effects of dopaminergic drugs. Psychopharmacology (Berl)., 232(21-22), 4043-4057. doi:10.1007/s00213-015-4009-8

[8] In order to create an extinction curve.

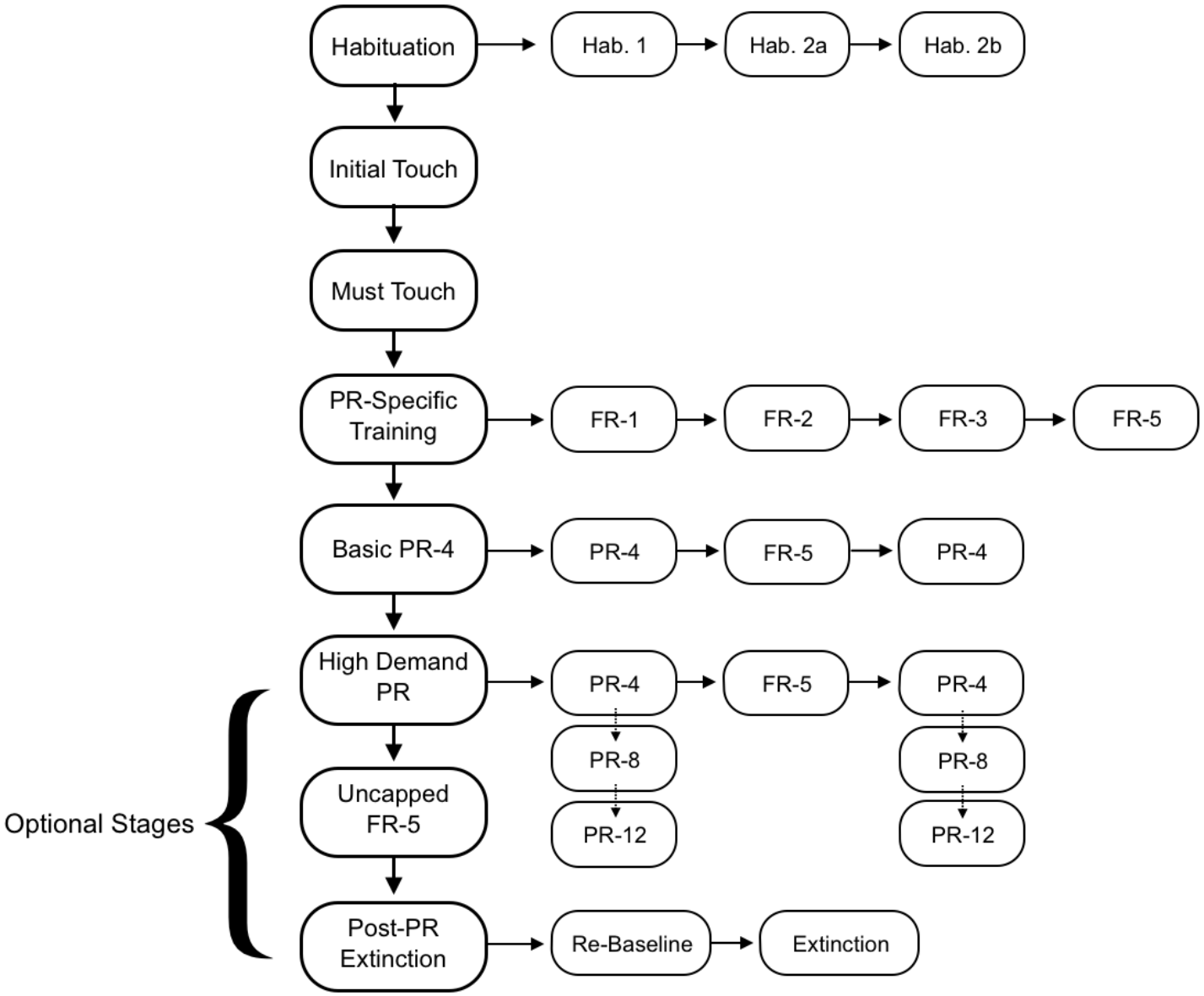
[9] If after 20 sessions (20 days) the mouse does not reach criterion for Extinction Learning, remove it from study. This includes the initial 10 days.

Heath, C. J., Phillips, B., Bussey, T. J., & Saksida, L. M. (2016). Measuring motivation and reward-related decision making in the rodent operant touchscreen system. Current Protocols in Neuroscience, 74(1), 8.34.1-8.34.20. doi:10.1002/0471142301.ns0834s74

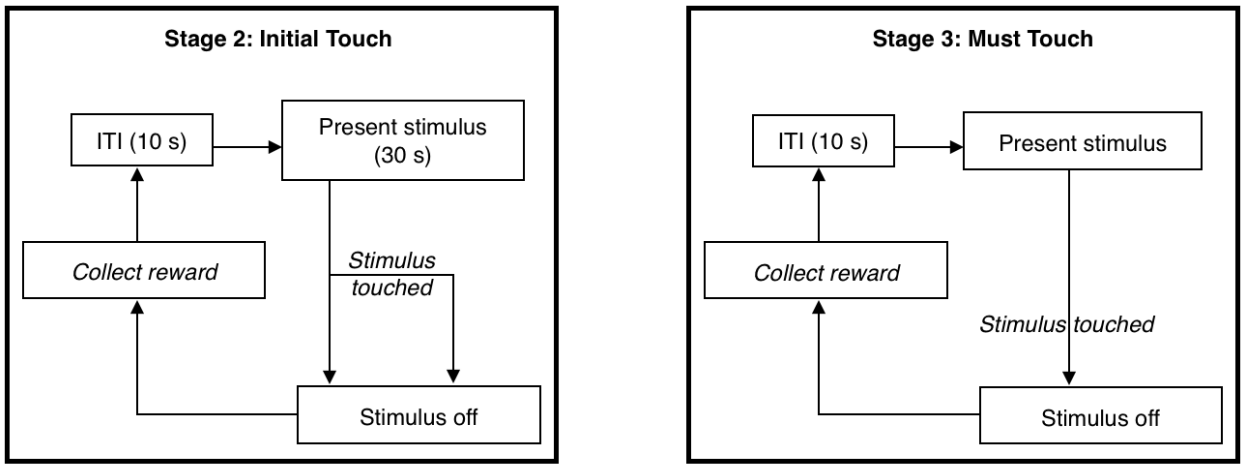
Heath, C. J., O'Callaghan, C., Mason, S. L., Phillips, B. U., Saksida, L. M., Robbins, T. W., … Sahakian, B. J. (2019). A touchscreen motivation assessment evaluated in Huntington's Disease patients and R6/1 model mice. Frontiers in Neurology, 10, 858. doi:10.3389/fneur.2019.00858

Mar, A. C., Horner, A. E., Nilsson, S. R. O., Alsiö, J., Kent, B. A., Kim, C. H.,… Bussey, T. J. (2013). The touchscreen operant platform for assessing executive function in rats and mice. Nature Protocols, 8(10), 1985-2005. doi:10.1038/nprot.2013.123

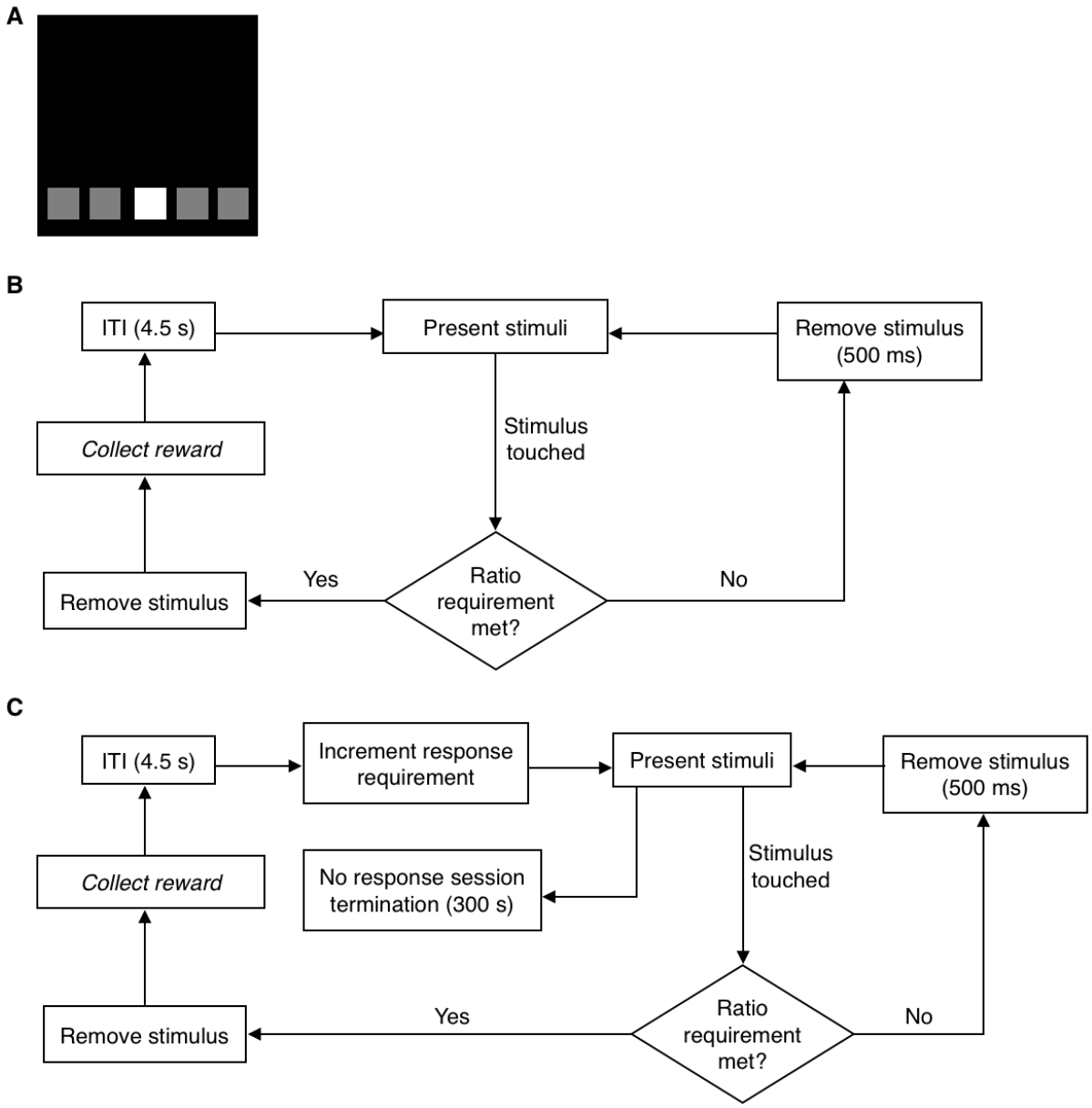
Zmavc, Katrina. (2019) The role of adult hippocampal neurogenesis in depression-relevant reward-related behaviours. MSc thesis; Western University Electronic Thesis and Dissertation Repository ID: 6734.



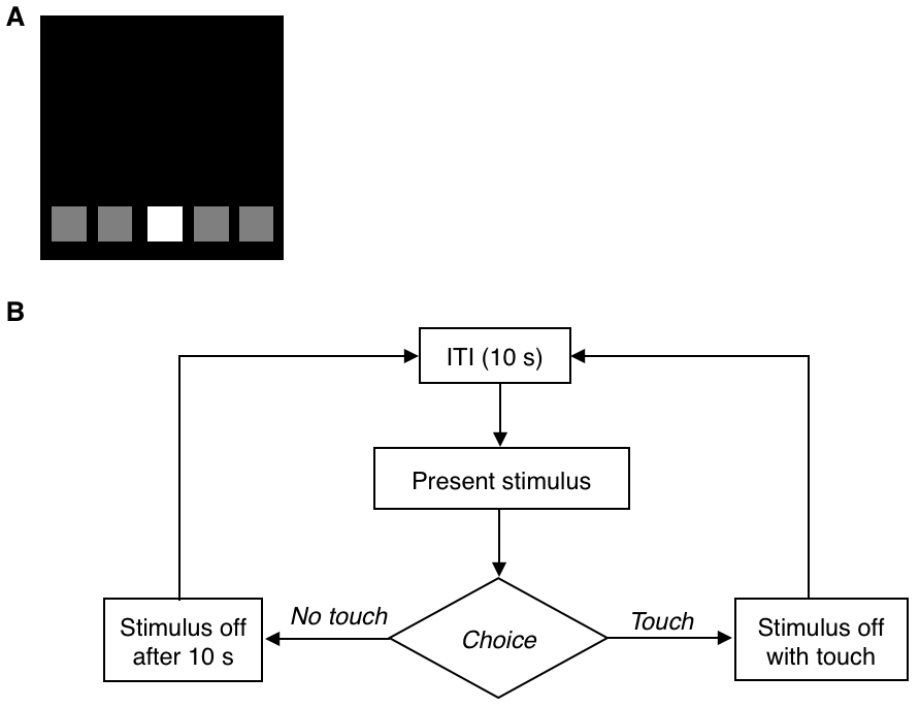
Flowchart of progressive ratio (PR) stages, including training and optional stages. Taken from K. Zmavc's MSc thesis.



Flowchart overview of Stages 2 and 3 of the basic pre-training listed in the SOP. Adapted from Fig. 1 in Mar et al., 2013; Taken from K. Zmavc's MSc thesis.



Schematic of the Fixed Ratio (FR) and Progressive Ratio (PR) test schedules described in the SOP. Adapted from Figs. 4 and 5 in Heath et al., 2016; Taken from K. Zmavc's MSc thesis.



Schematic of the Extinction learning test schedule described in the SOP. Adapted from Fig. 2(C) in Mar et al., 2013; Taken from K. Zmavc's MSc thesis.