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Dear Editors of JoVE,

On behalf of Abduselam Awol and myself, I submit our manuscript entitled "Kinase activity in the olfactory bulb is required for odor memory consolidation in mice" for your consideration.

Many studies have shown, using one-trial, fear-based paradigms, that post-learning *de novo* protein synthesis is necessary for long-term memory (LTM), but not short-term memory (STM). Further studies have gone onto examine the exact protein products differentially involved in LTM consolidation. Generalization of the findings to incremental, or multi-trial, learning can be problematic since incremental learning is by definition temporally distributed. Our manuscript outlines a simple and cost-effective rodent behavioral protocol that takes advantage of olfactory bulb (OB)-dependent associative memory. In the original Tong et al., 2018, Learn Mem study, we found using this protocol that mice that were given OB-specific infusions of tyrosine kinase receptor antagonist, K252a, immediately prior to training showed impaired memory when tested 48 hours, but not 2 hours, after training.

We believe that our manuscript fits well with JoVE's tradition of publishing methods of interest to readers of broad fields. Specifically, we hope that our manuscript will invite researchers with interests in the intracellular pathways in neuroscience to engage with the behaviors which those pathways underlie.

We have identified 3 reviewers that we feel would be qualified to critically review the work. Dr. Thomas Cleland whose work focuses on olfactory learning and memory, and Drs. AmyJo Stavnzer and Hewlet McFarlane who both work with rodent models in primarily undergraduate institutions.

I attest that this work is original. This manuscript has not been published elsewhere in part or in entirety, and is not under consideration by another journal. There are no conflicts of interest to declare, and all authors have read and agreed to submit our work to JoVE.

I believe that the findings of this study are relevant to the scope of your journal and will be of interest to your wide readership. I hope that our manuscript will find a home in your pages.

With appreciation,

Michelle T. Tong, PhD
Assistant Professor of Psychology
Neuroscience Program

TITLE:

A Low-Cost, Odor-Reward Association Task for Tests of Learning and Memory

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KEYWORDS:

Olfactory memory, kinase activity, memory consolidation, associative learning, discrimination task, olfactory bulb

SHORT ABSTRACT:

An odor-reward, associative learning task was used to investigate the differential effects of physiological manipulation on long-term and short-term memory.

LONG ABSTRACT:

Robust and simple behavioral paradigms of appetitive, associative memory are crucial for researchers interested in cellular and molecular mechanisms of memory. In this paper, an effective and low-cost mouse behavioral protocol is described for examining the effects of physiological manipulation (such as the infusion of pharmacological agents) on the learning rate and duration of odor-reward memory. Representative results are provided from a study examining the differential role of tyrosine kinase receptor activity in short-term (STM) and long-term memory (LTM). Male mice were conditioned to associate a reward (sugar pellet) with one of the two odors, and their memory for the association was tested 2 or 48 h later. Immediately prior to the training, a tyrosine kinase (Trk) receptor inhibitor or vehicle infusions were delivered into the olfactory bulb (OB). Although there was no effect of the infusion on the learning rate, blockade of the Trk receptors in the OB selectively impaired LTM (48 h), and not short-term memory (STM; 2 h). The LTM impairment was attributed to the diminished odor selectivity as measured by the length of the digging time. The culmination of the results of this experiment showed that Trk receptor activation in the OB is the key in olfactory memory consolidation.

INTRODUCTION

The mechanisms of associative memory formation have previously been investigated predominantly based on one-trial fear conditioning studies. However, many mundane tasks typically have more complex acquisition patterns and rely on repeated encounters. The goal of

this protocol is to provide a cost-effective rodent behavioral paradigm that is used to understand the cellular and molecular mechanisms of multi-trial appetitive learning and memory.

Odor learning that is dependent on the main olfactory bulb (OB) provides several advantages for the study of multi-trial appetitive memory. First, OB-dependent memories have varying durations (STM, LTM, and intermediate-term memory[1]) and rely on the same molecular[2,3] and structural mechanism as elsewhere in the brain, including neuromodulation[4], long-term potentiation[5], and adult neurogenesis[6-8]. Second, in contrast to the higher-order regions, like the hippocampus, OB-dependent memories allow for observations of a more direct correspondence between experimenter manipulations of the perceptual environment and changes to the neural circuitry responsible for learning[8-11]. In this paper, an OB-dependent associative learning and memory paradigm, that can be used to study general molecular and structural mechanisms, is detailed. It was developed to allow researchers to access the advantages of olfactory learning for the study of cellular and molecular mechanisms of memory.

In our recent publication[3], the protocol described here was used to demonstrate that the consolidation of appetitive odor learning is dependent upon Trk receptor activation within the OB. In the protocol below, areas where the behavioral paradigm can be adjusted for different experimental needs, are also discussed.

A total of 27 adult male CD-1 mice, 8 weeks old at the time of cannulation, were used in this study. For the precise group distributions and odor set use, see the methods section of our previous publication[3]. Male mice were used to avoid large fluctuations in estrogen levels because previous research[12] showed that olfactory memory is enhanced by increased estrogen levels. These mice were always kept on a 12:12 h reverse light/dark cycle and had access to water. During the behavioral experiments, the diets of the mice were restricted to maintain them at ~90% of their free feeding weight. Diet restriction began 3 days before the commencement of the behavioral experiment. As will be described below, the same set of mice are presented with different odor sets in order to reach the appropriate levels of statistical power while minimizing the animal use. The statistical analysis section shows how to account for the random variance that may be introduced by this.

PROTOCOL

The protocol below adheres to the animal care guidelines of the IACUC at Earlham College.

1. Olfactory bulb cannulation

NOTE: These surgeries do not require sterile technique since they do not require large incisions to be made. However, each institution may differ in their requirements. If experimenters are performing this surgery on immunocompromised mouse strains, additional considerations may be necessary. In all, experimenters are encouraged to discuss this protocol with their veterinarian and animal care team prior to use and to clean and disinfect all tools between each surgery.

1.1. During the setup, soak cannulae and screws in a small beaker or Petri dish containing 32% chlorhexidine to keep them disinfected.

1.2. Anesthetize mice with gaseous 4% isoflurane in pure oxygen and secure into a stereotaxic apparatus. During the surgery, ensure that the mice are maintained under 1.5-2% isoflurane anesthesia supplied through a nose cone. Monitor breathing throughout the surgery. Use eye ointment to prevent the dryness of eyes while under anesthesia.

1.3. After the mouse is secured and no longer responds to a firm pinch of the hind foot, use 32% chlorhexidine to rub the top of the head in order to clean incision surface.

1.4. Next, rub the top of the head in the rostral to caudal direction with lidocaine (topical analgesia).

1.5. Press down firmly to make a single incision down the midline with a clean scalpel blade.

1.6. Using a drill attached to the stereotaxic, drill two holes over the olfactory bulbs for the guide cannula (26 G) using coordinates AP +5.0 mm, ML +/-0.75 mm with respect to bregma.

1.7. Drill two holes over the cerebellar formation.

NOTE: The location of these screws does not need to be precise, ensure that they are symmetrical along the midline.

1.8. Place screws into the two holes over the cerebellar formation and use a tissue adhesive to secure these screws to the skull.

1.9. Use the stereotaxic to insert the guide cannula (26 G) into the holes drilled over the olfactory bulbs (step 1.6). Lower the cannula DV 1.0 mm.

1.10. Mix the dental cement in a Petri dish. Use a small metal scoop to slowly pile the dental cement around the cannula. Let it dry for 5 s. Then, remove the arms of the stereotaxic device, being careful not to pull the guide cannula out in the process. Continue piling the dental cement over the entire incision until a small cap is formed (See **Figure 1A**).

1.11. Place dummy plugs into the guide cannula at this point to prevent the blockage.

1.12. Immediately after the surgery, inject ketoprofen (0.2 mg/kg) and saline (200 µL) to reduce pain and rehydrate. Provide softened food or hydrogel to the mice after surgery. Do not leave mice unattended until they have regained consciousness to maintain sternal recumbency.

NOTE: Mice are also singly housed from this point forward.

1.13. Inject saline (200 µL) and ketoprofen (0.2 mg/kg) once a day for two days after the surgery.

1.14. For 2 days and up to 5 days, (as needed) after the surgery, weigh the mice and monitor their weights. If the weight does not return to pre-surgery levels within two or three days, consult with the veterinarian about the appropriate method of feeding.

1.15. Allow mice to recover for at least 7 days before beginning the behavioral training.

2. Associative discrimination task

2.1. Infusions

2.1.1. Administer OB-specific infusions of the tyrosine kinase receptor inhibitor, K252a (50 µM; 5% DMSO in saline), or vehicle (5% DMSO in saline) to the mice as follows.

2.1.1.1. Deliver the infusion into the OB of the mice bilaterally. Inject 2.0 µL final volume per bulb with 0.2 µL/min infusion rate and 10 min of total infusion time using a dual injector pump.

NOTE: Timing of the manipulation can be adjusted depending on the type of behavioral study being performed. Exact steps of infusion are specific to each injector pump and provided in the manufacturer's manual.

2.1.1.2. Make sure to leave the injectors inside the cannulae for about 5 min after delivery to inhibit the backflow and promote diffusion. (i.e., plan for a total of 15 min for each infusion).

2.2. Odor sets.

2.2.1. Dilute all odorants in light mineral oil to a partial pressure of 1.0 Pa using a pre-calculated ratio based on vapor pressure (**Table 1**).

2.2.2. Use the 5 separate odor pairs from **Table 1** (numbers in the table indicate volume in µL to mix in 50 mL mineral oil for 1.0 Pa).

2.2.3. To prepare the scented sand to be used during the behavioral steps (Section 2.3 and 3), mix 400 µL of the 1.0 Pa odorant from Step 2.2.2 for every 100 g of play sand.

2.3. Shaping

NOTE: Mice should undergo shaping for a period of 10 days as described below.

2.3.1 Bring mice into the procedure room and handle them for 10 min per day for the first two days following recovery from surgery.

2.3.2 On Day 3, place a Petri dish filled with (+)-limonene scented sand into the home cages of the mice and filled with about 10 sucrose pellets, each 5-mg in mass.

NOTE: Use 1.0 Pa (+)-limonene (mix 102 µL in 50 mL mineral oil) as the rewarded odor and plain mineral oil (the diluting agent for the testing odors) as the unrewarded odor. Choosing monomolecular odorants is also recommended since these are most likely to be novel to mice.

2.3.3 Replenish both the sand and the pellets on Day 4.

2.3.4 On Days 5 and 6, acclimate the mice to the custom-made behavioral apparatus, by placing them into the apparatus and letting them explore the space (**Figure 1B,C**). Make the apparatus using a standard home cage, and poly (methyl methacrylate) to construct two lids and a black center divider. Ensure that both the lids and the center divider are 1-2 cm larger than the home cage.

2.3.5 Prepare one Petri dish of limonene scented sand and another with sand containing mineral oil. After acclimation on both Day 5 and 6, place both the dishes of sand into the test chamber and mix 10 sucrose pellets into the limonene-scented dish to serve as the reward. Place each mouse into the test chamber for 10 min and allow to freely explore and consume the reward pellets.

2.3.6 On Day 7, introduce mice to an abridged version of the final testing procedure, by placing the dishes containing limonene scented and mineral oil scented sand into the behavioral apparatus. This time include the center divider.

2.3.6.1 Place a single reward on top of the limonene-scented sand and place the mouse into the resting chamber.

2.3.6.2 Once the mouse has been placed in the resting chamber, lift the center divider to allow the mouse to enter the test chamber to investigate and dig in the sand-filled dishes. Return the mouse to the resting chamber either after it has retrieved the reward pellet or after 5 min has elapsed.

2.3.6.3 Repeat this process for a total of 10 trials for each individual mouse. Counterbalance the placement of the rewarded dish of either on the left side or right side by employing a random number generator. There are no rest periods between trials.

2.3.7 On Day 8, repeat the trials of Day 7, but also progressively bury the pellet deeper and deeper in the sand.

NOTE: Most mice should be digging for the unseen reward pellet by the 10^{th} trial on Day 8.

2.3.8 On Day 9, increase the number of trials to 20 trials for each mouse with the full deep burial of sucrose pellet and introducing the mice to the testing chamber for 1 min per trial. Let mice dig in both dishes for the reward.

2.3.9 On Day 10, repeat the 20 trials for each mouse, but if they dig in the unrewarded dish before they dig in the rewarded dish, and then begin the next trial. Allow the mice that first dug in the rewarded (limonene-scented) dish to retrieve the reward pellet before sending them back to the resting chamber.

3. Training and testing

NOTE: Once the mice have started digging reliably for the unseen, odor-cued reward pellets, the experiment can commence.

3.1. Training

NOTE: The training phase begins two days after shaping is completed and consists of 20 trials for each mouse. Prior to the training**,** deliver the intrabulbar drug/vehicle infusions immediately (see Section 2.1 for infusion details) and begin the training immediately following the infusions.

3.1.1 Place a mouse into the resting chamber.

3.1.2 Place two dishes of sand scented with a novel odor-pair in the test chamber, where a reward pellet is buried in one of the dishes.

3.1.3 Once the testing chamber is ready, lift the opaque barrier and introduce the mouse into the test chamber. Immediately return the mouse to the resting chamber, if the mouse digs in the unrewarded dish first (record these trials as a "0"). If the mouse digs in the rewarding odor first, allow it to retrieve the pellet and return it to the resting chamber. Record these trials as a "1." If the trial lasts 1 min without the mouse retrieving the reward, send the mouse back to the resting chamber.

3.1.4 Clean and refill dishes and begin the next trial. Repeat the same for 20 trials.

3.2. Testing

NOTE: Memory testing can be performed at any duration of interest to the researcher. In this experiment, two separate groups of mice were tested 2 h (STM) or 48 h (LTM) following the training given the interest in the differential influence of K252a on STM and LTM.

3.2.1 Carry out testing using the same odors and procedure as described for the training (Section 3.1).

NOTE: Depending on the research question, the researcher may need to include control groups. For example, in the previously published experiment, the effects of Trk receptor blockade on memory consolidation was studied[3]. Therefore, a control group was infused with K252a prior to 48-h testing to show that the effects were not due to interference with retrieval.

3.3. Statistical analysis

NOTE: SPSS 22.0 syntax for each step provided as a **Supplemental File** as an example.

3.3.1 Perform statistical analysis using linear mixed effects analysis. This, unlike ANOVA, can account for random effects.

3.3.2 Calculate the dependent measure: "proportion correct." Recall from 3.1.3 that a "1" was assigned to trials in which the mouse dug in the rewarding odor first, and a "0" if the mouse dug first in the unrewarding odor. Average every five trials to create four trial blocks (TB; e.g., trial block 1 or TB1 was the average of trials 1-5, trial block 2 or TB 2 was the average of trials 6-10 and so forth).

3.3.3 Set the independent variables or fixed effects as drug groups (K252a or Vehicle; Section 2.1.1) and trial blocks (from 3.3.1). In the representative results below, the variables used for each analysis are specified.

3.3.4 Include individual mouse and odor set nested within the mouse as "random effects" in order to compensate for intrinsic behavioral differences in the mice and any effects of using multiple odor sets.

3.3.5 Perform a logit transformation on the proportion correct.

NOTE: The proportion correct is not a continuous, unbound dependent variable. Thus, it violates two assumptions for linear models. Therefore, the logit transformation is performed.

3.3.6 Use estimated marginal means to perform post hoc tests on significant interactions identified by the full model; multiple pairwise comparisons must be corrected for in post hoc testing. Bonferroni or Šidák are typically used.

REPRESENTATIVE RESULTS

As described, this protocol allows researchers to assess the influence of some manipulation on learning, STM, and LTM. Sample results from Tong et al, 2018[3] are presented here. The results support the hypothesis that Trk receptor blockade selectively inhibits LTM, but not learning or STM.

Figure 2A shows the schematics of training, STM test, and LTM test. First, it was shown that K252a infusions did not affect the learning rate of an odor-reward association. **Figure 2B** (Section 3.1) shows the learning rate of both K252a and vehicle groups from Training. Statistical analysis using a linear mixed model was run with two fixed effects, drug group and trial block (TB); mouse and odor set nested within the mouse were random effects. Data from Testing (Section 3.2) were not included in the analysis. A significant main effect was seen from trial block ($F(3, 183.692) = 43.735$, $p < 0.001$), but no effect from drug group ($F(1, 85.685) = 0.132$, $p = 0.717$) and no significant interaction ($F(3, 183.692) = 0.111$, $p = 0.954$). Post hoc tests, using the Šidák

adjustment, confirmed that the K252a and vehicle groups did not differ on any of the trial blocks during Training ($p > 0.05$ for all comparisons). TB2, TB3, and TB4 were significantly higher than TB1 for all comparisons ($p \leq 0.001$ in all cases), demonstrating that both groups successfully learned the odor-reward association by the end of 20 Training trials.

Next, to examine the effects of the infusion on STM and LTM, the same analysis was run and included data from Testing (Section 3.2). It showed a significant interaction between drug group and trial block ($F(2, 77.558) = 4.043$, $p = 0.021$), with no significant main effects of drug group ($F(1, 55.629) = 1.438$, $p = 0.236$) or trial block ($F(2, 69.979) = 1.360$, $p = 0.263$). In order to specifically examine memory, post hoc pairwise comparisons with Šidák correction, compared differences between the last trial block of Training (Section 3.1) and the first trial block of Testing (Section 3.2) either 2 (STM) or 48 hours (LTM) later. For vehicle-infused mice, the comparisons showed retention of the associative memory at both 2- and 48 h post-training ($p > 0.05$ for all comparisons with training performance). For K252a-infused mice, the first trial block of the 2-hour test (STM) did not differ from the last trial block of Training ($p > 0.05$); however, their memory performance was significantly lower after 48 h ($p = 0.018$). In addition, memory at the 48-hour test was significantly reduced compared to memory at the 2 hours test ($p = 0.009$), and to the performance of the vehicle group at the 48-hour test ($p = 0.006$). There was no difference in STM between vehicle- and K252a-infused mice ($p = 0.356$). Together, the results show that K252a inhibition of Trk receptors in the olfactory bulb selectively disrupts long-term, but not short-term, odor memory (**Figure 3**).

FIGURE LEGENDS

Table 1: Mixing volumes for odor sets. Each row shows two odors that can be used as a pair for the behavioral steps. For example, to use the first "odor set," make the pentanoic and butanoic acid mixtures. The numbers in the table indicate volume in µL to mix in 50 mL mineral oil for 1.0 Pa concentration of each odor. During training and testing, one Petri dish would be scented with pentanoic acid, the other with butanoic acid.

Figure 1: Cannulation placement and behavioral apparatus. (**A**) Shows the relative position of the cannula, dental cement cap, and screws to the head of the mouse. Note that the needles of the cannula reach into the two olfactory bulbs, the pedestal itself is embedded into the dental cement cap. The screws are placed into two holes drilled into the skull over the cerebellar formation. The screws do not touch the brain itself, but they act as a caudal anchor for the dental cement cap. The figure shows the relative size to make the cement cap. (**B**) Shows the assembled behavioral apparatus. The body is a typical mouse home cage. Lids with holes for air were made from plexiglass. The center divider is also made from black plexiglass. The lids should be made from heavy enough plexiglass that they can act as a tract for the center divider to be lifted and placed down during the trials. (**C**) Shows one side of the behavioral apparatus. Note that the center divider is slightly taller than the cage for ease of lifting. Petri dishes of sand can be placed along the edge.

Figure 2: Study design and learning results. (**A**) Shows the schematic of the study design. Note

that the STM and LTM groups were independent (i.e. different groups of mice). The symbols at the beginning of the blocks indicate the time when infusions were given. (**B**) Shows the proportion correct for Trials 1-20 during Training. The results indicate that K252a and vehicle groups did not differ in their learning rate (slope of the lines). Error bars represented the standard error of the mean (SEM). Asterisks show significant increases in proportion correct compared to TB1 for both vehicle and K252a cohorts ($p \leq 0.001$ for all comparisons). This figure is adapted from Tong et al. 2018 with permission[3].

Figure 3: Differential effects of Trk receptor blockade on STM and LTM. Shows the proportion correct for Trial Block 4 of Training and Trial Block 1 of both the STM and LTM Testing. That is, a linear mixed model is used to compare the proportion correct during the last trial block of the training phase (Figure 2; Training-TB4) to those during the first trial blocks (Testing-TB1) of short-term (2-hours test) and long-term (48-hour test) memory test. The linear mixed model had two fixed effects: drug group and trial block (Training-TB4, STM-TB1, LTM-TB1). The random effects were mouse and odor set nested within the mouse. Post hoc comparisons found that K252a mice had significantly impaired LTM (comparison with training TB4; $p = 0.018$) but not STM ($p > 0.05$). LTM performance by K252a-infused mice was also significantly lower than the STM of K252a-infused mice ($p = 0.009$), and lower than LTM of vehicle mice ($p = 0.006$). Error bars represented the SEM. This figure is adapted from Tong et al. 2018 with permission[3].

Supplemental File: Syntax used for the statistical analysis.

DISCUSSION

One-trial fear conditioning is a powerful behavioral protocol for studying molecular and cellular dynamics of memory, but much of natural learning is incremental and best modeled through a paradigm like the one described above. The inhibition of Trk receptors in the OB prevented the consolidation of olfactory memory in a multi-trial, appetitive learning paradigm as shown previously by our group[3]. The finding opens new avenues for research into the differential timing of molecular mechanisms, like neurotrophins, in appetitive and aversive learning.

This experiment consisted of two critical parts: (1) the cannulation and (2) the associative discrimination task (subdivided into shaping, training, and testing). Experimenters can adapt this protocol to their specific research question. For example, we were primarily interested in the OB and this established protocol can be easily applied to other OB studies. For experimenters with other regions of interest, it will be important to validate the infusion sites in a pilot study. Experimenters may also need to consider the diffusion rate, spatial penetration, and bio-activity duration of what they are infusing.

The shaping steps outlined in the protocol have been used extensively by the authors of this protocol. Adhering to them as described appears to be important to have the mice learn the task in a timely manner. Using other timelines, the authors observed more variance amongst the mice in their familiarity with the task and this meant additional training to get all mice to a criterion for testing with experimental odors. For training and testing, the researcher has flexibility, depending on their research interests, with the number of trials, the concentration of the

experimental odors, and the similarity of the odors to one another. We recommend the use of multiple odor sets when possible, as we've described, in order to reduce the number of animals used for the experiment. See the Statistical Analysis section for the guidance on how to account for the use of multiple order sets in the final analysis. In principle, the similarity of the odor pair can be varied to adjust the difficulty of the discrimination. In the previously published study from our group[3], the odor pairs consist of two odorants of the same functional group but differed from each other by one carbon length. These discriminations are more difficult than pairs that differ by two or more carbon lengths, but easier than enantiomers (e.g., (+)-limonene and (-)-limonene). Odorants from different functional groups are highly different perceptually. Cleland et al[13] discusses more stimulus variations and their effect on specific learning parameters.

One major limitation of this protocol is that it takes a much longer time to carry out compared to automated associative learning tasks where multiple animals could be tested in parallel. For a given test, it would take one researcher at least 20 minutes to complete 20 trials for one mouse. However, it is this lack of automation that means the protocol is more financially accessible, a priority for many institutions. Importantly, in the case of this experiment, it has been found that this protocol is highly tractable and effective for training undergraduate researchers with interests in behavioral neuroscience. In particular, these students develop strong animal handling skills in addition to the usual benefits of research participation.

Researchers who are interested in adopting this paradigm can vary several parameters. Most apparently, pharmacological manipulations of mechanisms are diverse, and this behavioral protocol can be used with chemogenetic techniques or various other ways of manipulating molecular and cellular pathways (e.g. optogenetics). The paradigm itself can be adjusted to vary the kind of learning and memory tested. For example, researchers can adjust the similarity of the two odors presented in order to control the learning rate. In our study[3], the odor pairs consist of two odorants of the same functional group but differed from each other by one carbon length. These discriminations are more difficult than pairs that differ by two or more carbon lengths, but easier than enantiomers (e.g. (+)-limonene and (-)-limonene). Odorants from different functional groups are highly different perceptually. Cleland et al[13] discusses more stimulus variations and their effect on specific learning parameters, concluding that more similar odors are more difficult to discriminate and therefore take longer to learn[14]. These manipulations would also affect the strength of memories. Along this vein, researchers may be interested in testing memory at different timepoints after learning. For example, two studies[15,16] examined the role of BDNF in LTM persistence for a one-trial aversive learning task. BDNF is a ligand of TrkB. The studies showed that anti-BDNF antisense oligonucleotide infusion in the hippocampus 12 hours after learning blocked LTM 7 days later, but not 2 days later. This study shows that the timescale of molecular mechanisms after initial learning play interesting, and yet-to-be-understood, roles in LTM. This paper describes a behavioral protocol that would allow for the investigation of these timescales. Other parameters of interest for future application include the mouse model used. For example, it would be interesting to replace male mice (who have a significantly better studied neurobiology)[17] with female mice in future studies to examine variations in the learning rate, STM, and LTM, as female mammals have a higher sensitivity and selectivity to odors than male mammals[18]. Of course, rodent models of diseases can also be used effectively with this protocol.

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DISCLOSURE

The authors of this paper had no competing financial interests.

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Figure 1 (New)

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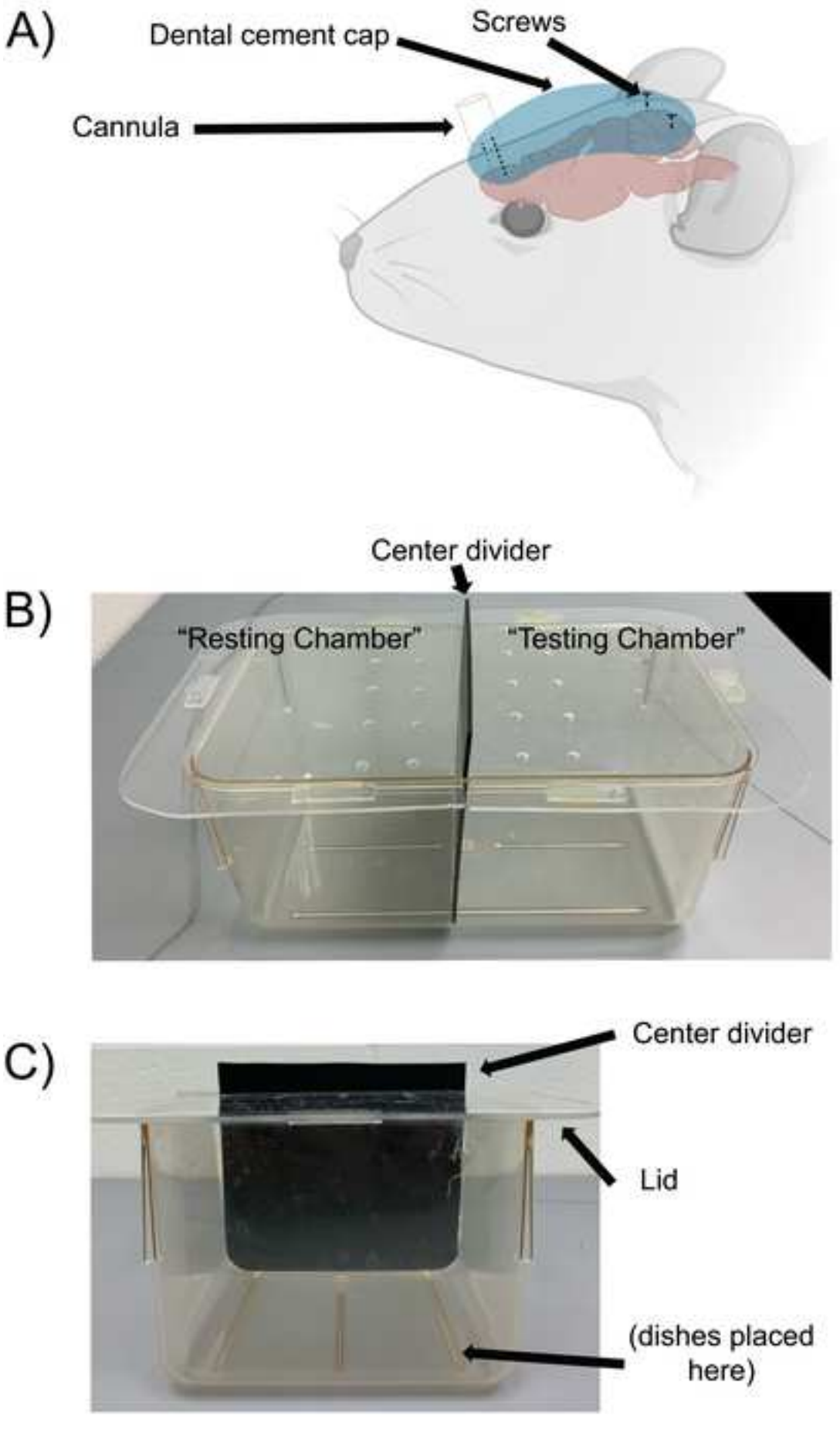

Figure 2 (Previously Figure 1)

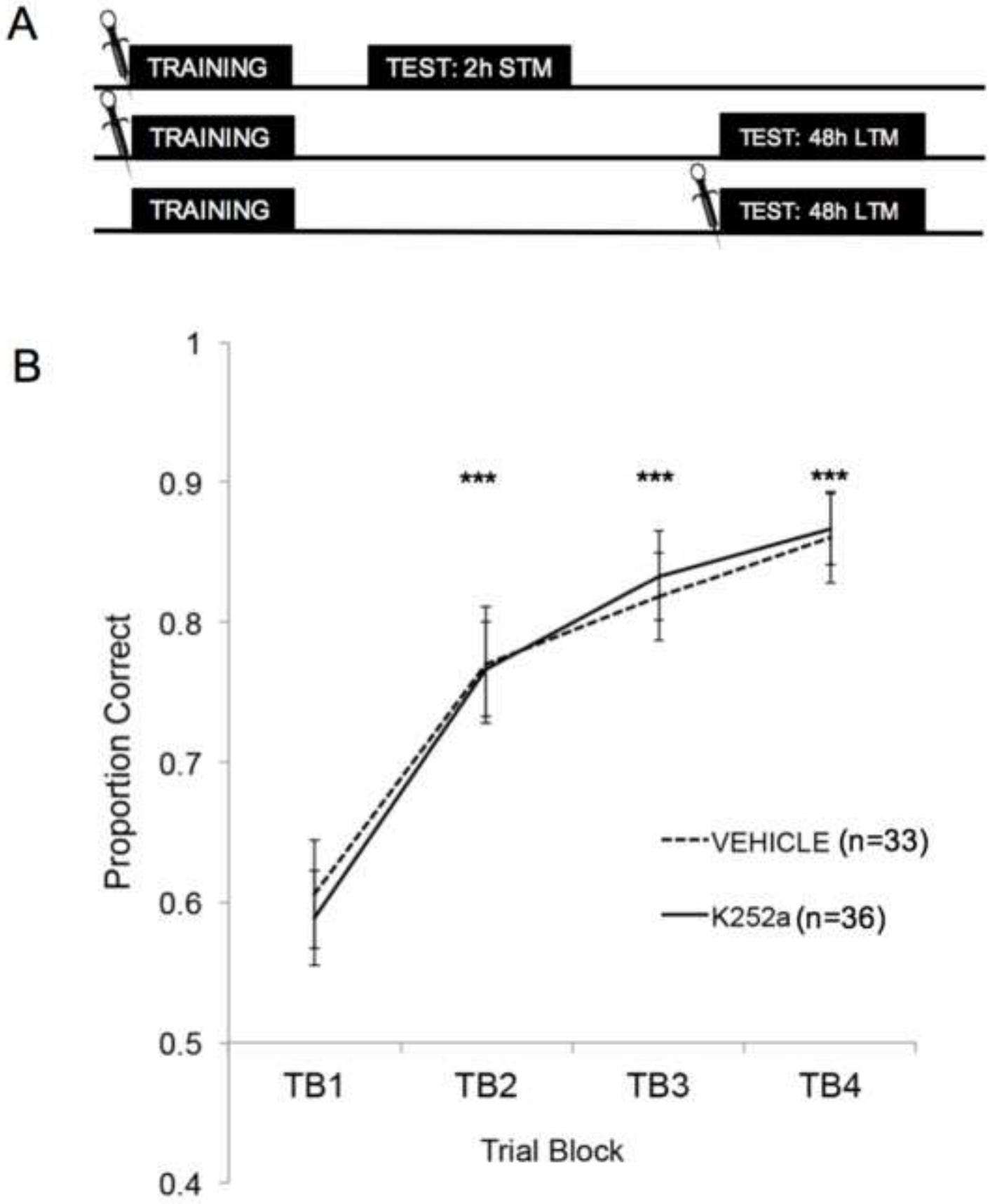

Figure 3 (Previously Figure 2)

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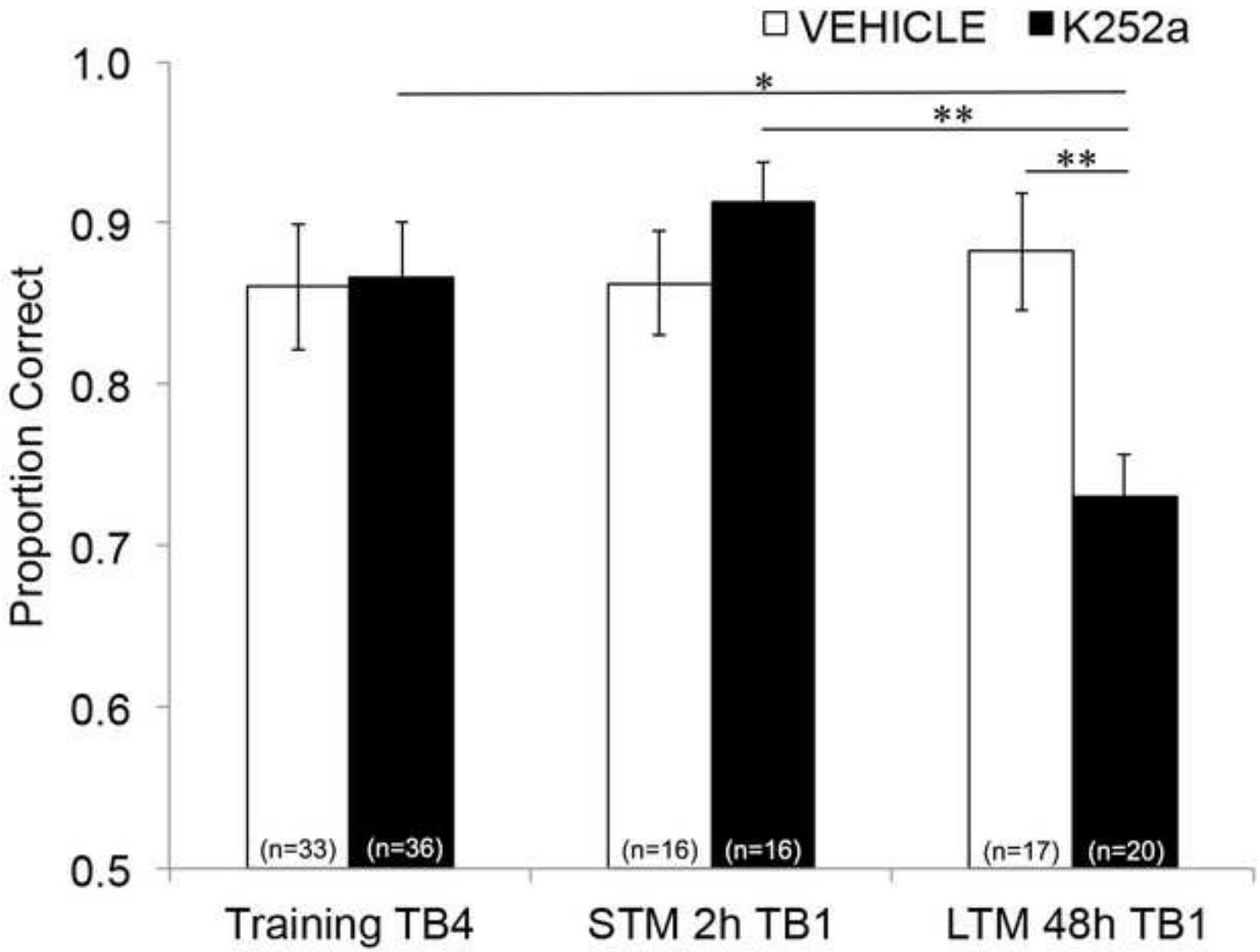

Table 1

Odor set	Odor 1	Odor 2
1	pentanoic acid 225.1	butanoic acid 63.6
2	hexanal 11.1	heptanal 35.3
3	propyl acetate 3.1	butyl acetate 10.9
4	2-octanone 87.4	2-heptanone 28.7
5	pentanol 37.2	hexanol 127.3

Name of Material/Equipment	Company	Catalog Number
Double guide cannula	PlasticsOne	C235GS-5-1.5/SPC
(-)-limonene	Sigma-Aldrich	218367-50G
(+)-limonene	Sigma-Aldrich	183164-100ML
2-hetanone	Sigma-Aldrich	537683
2-octanone	Sigma-Aldrich	O4709
5mg sucrose pellets	Test Diet	1811560
Butanoic acid	Sigma-Aldrich	B103500
butyl acetate	Sigma-Aldrich	402842
Dental Cement Powder (Coral)	A-M Systems	525000
Dental Cement Solvent	A-M Systems	526000
Double connector assembly	PlasticsOne	C232C
Double dummy cannula	PlasticsOne	C235DCS-5/SPC dummy dbl
Double injector	PlasticsOne	C235IS-5/SPC
Drill	Kopf Instruments	Model 1474 High Speed St
Eye Ointment		
Figure 1 illustration software	BioRender	
heptanal	Sigma-Aldrich	W254002
hexanal	Sigma-Aldrich	115606
hexanol	Sigma-Aldrich	H13303
Infusion pump model 11	Harvard Apparatus	4169D
Isoflurane	Santa Cruz Animal H	sc-363629Rx
K252a	Sigma-Aldrich	K2015
Ketoprofen	Allivet	25920
Lidocaine	Aspercreme	
Mounting Screws	PlasticsOne	00-96 X 3/32
Mouse Anesthesia Mask	Kopf Instruments	Model 907 Mouse Anesth
Mouse Nose Adaptor	Kopf Instruments	Model 926 Mouse Adapto
Novalsan	Jeffers	41375
Pentanoic acid	Sigma-Aldrich	240370
pentanol	Sigma-Aldrich	138975
Petri dish glass bottoms	VWR	10754-804

Polycarbonate Café bottoms	Ancare	N10PCSEC
propyl acetate	Sigma-Aldrich	537438
Quikrete Premium Play Sand		
Saline	Insight Needles	N/A
Stereotaxic apparatus	Kopf Instruments	Model 902 Small Animal S
Testing chamber	Ancare	N10PCSEC
Vetbond Tissue Adhesive	3M	

Comments/Description
Custom order
Custom size. Used for rewards
Custom order
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This drill requires an additional "adaptor" piece in order to fit certain drill bits. We get by this problem by wrapping the drill bit with
Purchase from local pharmacy
Used pumps available via American Instrument Exchange
Vet prescription needed for order
Mixed to 50uM in DMSO (5%)
Vet prescription needed for order
Purchased from Amazon
Used with the stereotaxic to allow oxygen and anesthesia while mouse in stereotax
Used with the stereotaxic to allow for head of mouse to be secured.

Use normal housing cages and custom fit a track in the middle to act as the track for an opaque plexiglass divider

Purchase from local hardware store
Sterile saline for drug mixing
tereotaxic Instrument
Our testing chambers are modified using the regular mouse housing cage. The manuscript details what was done.
Purchased from Amazon

ı lab tape to increase the circumference of the drill it to fit. This may not be an option for surgeries requiring sterile technique.

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Author(s):	Abdusalem K. Awol and Michelle T. Tong

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CORRESPONDING AUTHOR

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Responses to Editorial and Reviewer Comments for Awol et al.

We thank the editor(s) and reviewers for their time, careful review, and helpful feedback. We have rewritten the reviewer's responses below (in normal face type), and have responded to each of the comments (in green italic type).

Editorial comments

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
2. Please ensure that the manuscript is formatted according to JoVE guidelines–letter (8.5" x 11") page size, 1-inch margins, 12 pt Calibri font throughout, all text aligned to the left margin, single spacing within paragraphs, and spaces between all paragraphs and protocol steps/substeps.
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- We revised the manuscript according to the general comments (1-5) above.

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- Completed.

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d) Discuss maintenance of sterile conditions during survival surgery.
e) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.
f) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

- We revised the manuscript according to comments (a)-(f) above.

3. There is a 10 page limit for the Protocol, but there is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headers and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

- Completed.

4. For each protocol step/substep, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

- Completed.

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- I uploaded the email correspondence with the editor about this.

2. Please cite the Figures within the manuscript itself (outside the Figure legends).

- Completed.

3. Figure 1A: What are the symbols at the start of training/test?

- Thank you for pointing out the need for clarification. We added an additional line (underlined in the quoted text below) in the figure caption to provide further explanation. Note that Figure 1A is now Figure 2A (with the addition of photographs)

> "(A) Shows the schematic of the entire design of the Tong et al., 2018 study. Note that the STM and LTM groups were independent (i.e. different groups of mice). The symbols at the beginning of the blocks indicate the time when infusions were given."

4. Figure 1B: What are the error bars and stars??
5. What do the stars represent?

- Thank you for the two questions in 4 and 5 above. We added these additional lines (underlined in the quoted text below) in the figure caption for both figures to provide further explanation. Note that Figure 1 is now Figure 2 (with the addition of photographs)

Figure 2 “(B) Shows the proportion correct for Trials 1-20 during Training. The results indicate that K252a and vehicle groups did not differ in their learning rate (slope of the lines). Error bars represented the standard error of the mean (SEM). Asterisks show significant increases in proportion correct compared to TB1 for both vehicle and K252a cohorts ($p \leq 0.001$ for all comparisons). Figure from Tong et al., 2018.”

Figure 3 “The random effects were mouse and odor set nested within the mouse. Post hoc comparisons found that K252a mice had significantly impaired LTM (comparison with training TB4; $p = 0.018$) but not STM ($p > 0.05$). LTM performance by K252a-infused mice was also significantly lower than the STM of K252a-infused mice ($p = 0.009$), and lower than LTM of vehicle mice ($p = 0.006$). Error bars represented the SEM. Figure from Tong et al., 2018.”

Discussion:

1. Please revise the Discussion to explicitly cover the following in detail in 3–6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique

- Thank you for these questions. We made substantial edits in response to these points so we ask the editor and reviewer to read the Discussion section to see the changes.

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1. Please include an Acknowledgements section, containing any acknowledgments and all funding sources for this work.
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- We added information for both Acknowledgments and Disclosures.

References:

1. Please number references in the order in which they appear in the manuscript.

- Completed.

Table of Materials:

1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

- Thanks for pointing this out. Please see the updated Table of Materials.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:
To understand cellular and molecular mechanisms of memory, the authors describe an effective mouse behavioral protocol for examining the effects of physiological manipulations on olfactory bulb-dependent short-term and long-term memories.

- We thank the reviewer for this clear summary of the paper.

Reviewer #2:

Manuscript Summary:

In this manuscript authors described a new behavioral protocol to test learning rate and odor-reward memory. This protocol is cheap and effective, constituting a new alternative in order to analyze those behaviors. It is specially interesting as an easy way to implement odor behavior in labs with difficulties in funding.

Major Concerns:

No major concerns were detected in the manuscript. It is true that the protocol described is long and it is required personal for longer periods than other behavioral tests, but authors already pointed that and as I described above it could be a good approach for new researchers who are interesting in this field in a low cost and easy way.

- Thank you for your acknowledgement here. The trade-off you articulated here is exactly what advantages this protocol for use in training new researchers.

Minor Concerns:

I missed more explanation about why they choose to use males, and why females could behave differently. This is only mentioned at the end of author´s discussion in lines 339-341.

- Thank you for pointing out the need for clarification. We added an additional line (underlined in the quoted text below) in the Introduction to provide further explanation:

  > "In Tong et al., 2018, a total of 27 adult male CD-1 mice, 8 weeks old at the time of cannulation, were used in this study. For the precise group distributions and odor set use, see the methods section of Tong et al., 2018. Male mice were used to avoid large fluctuations in estrogen levels because previous research (Dillon et la., 2013) showed that olfactory memory is enhanced by increased estrogen levels. These mice were kept on a 12:12 hour reverse light/dark cycle …."

I wonder if they have consider the litter effect in the mice model used, when they have performed the statistical analysis. If so, can they described how they took that into consideration?

- This is a great point! We did not account for a potential litter effect in Tong et al., 2018. We order same-aged mice directly from a vendor, and do not typically know how many litters the mice come from. In the future, it would be possible for us to either ask for mice from same litter or ask the vendor to provide this information for the mice. In the linear mixed effects analysis, we could add litter as a potential random effect (similar to what we do for odor set and mouse).

It is confusing of how mice were distributed into the different groups. Could they improved the protocol description (from line 71) in order to get a clear idea of how many mice were used for each group. In accordance with my previous comment, figure 1 should be modify in order to be clear. Please provide the number of mice used for each group.

- Thank you for this insight. We were intentional vague about Tong et al., 2018 details since it felt that the experimenters adapting this protocol should determine their own

numbers. However, we understand that the specifics could be helpful as an example. We've added the following direction for the reader (underlined):

> "In Tong et al., 2018, a total of 27 adult male CD-1 mice, 8 weeks old at the time of cannulation, were used in this study. For the precise group distributions and odor set use, see the methods section of Tong et al., 2018. Male mice were used to avoid large fluctuations in estrogen levels because previous research (Dillon et la., 2013) showed that olfactory memory is enhanced by increased estrogen levels. These mice were kept on a 12:12 hour reverse …"

In all graphs, each mice should be represented as a point. This gives a better understanding of mice behavior, instead of just pointed error bars.

- Thanks so much for this comment. I was just reading some articles about this practice, and am in whole-hearted support of it. In fact, we're doing this right now with our figures for another manuscript. For the sake of the current JoVE paper, we plan to leave the graphs as is to allow easy comparison between the representative results and the original Tong et al., 2018 paper that they were drawn from.

Reviewer #3:

Manuscript Summary:

This manuscript describes an elegant method to analyze the effect of tyrosine kinase receptor activity on learning rate and odor-reward memory using pharmacological manipulation via cannula infusion in the mouse olfactory bulb. The experiment presented here reveals the differential role of tyrosine kinase receptor activity in short and long-term memory on the behavioral level. The protocol presented by the authors is clearly written and the steps are easy to follow. The detailed description of the statistical analysis is useful for understanding and interpreting results. This comprehensive and elegant approach is ideally suited for the video format provided by JoVE.

- Thank you for your kind comments and accurate summary.

A few points that will improve the manuscript and its usefulness for the JoVE platform:

Major Concerns:

1) A schematic of a mouse head illustrating how the cannulas are implanted and how they are fixed to the skull will greatly facilitate the reproduction of the surgery.
2) The manuscript would benefit from a photograph or detailed schematic of the animal cage with the custom divider. More instructions on how to build/source the cage divider will be useful as well.

- Thanks for the insights in Concern 1) and 2). We agree, and we've added a new Figure 1 that shows a diagram of the mouse head after cannulation surgery and some photos of the components of the behavioral apparatus.

3) A video of the behavioral task with the implanted animal being placed in the cage would provide helpful information about the experiment.

- Thank you for this comment. We'll be mindful of this when we work on the video.

Minor Concerns:

4) Line 107: The final concentration of 5% DMSO to solve K252a and for the vehicle is high and has been shown to have toxic effects and even induce apoptosis in vivo (e.g. Lin et al., Toxicol. Appl. Pharmacol, 2015). This does not interfere with the description of the method but it possibly has an effect on cell viability.

- Thank you for this reference.

5) Line 131: replace solvent with 'diluting agent'

- Thank you. We made the replacement.

6) Line 133: Please point out what the calculation is based on. Vapor pressure? This information will be necessary when reproducing the experiment with different odors.

- We edited the text to clarify this.

7) Line 159: Please describe the volume of odor and amount of sand to fill the dish with.

- Thank you for catching this. We've added the text below to Section 2.2:

  "2.2.4. To prepare scented sand for behavioral trials below, mix 400 uL of the 1.0Pa odorant for every 100g of play sand."

8) Line 189-195: Please rephrase. The procedure of sending back the animal after digging in the unrewarded dish is described in a repetitive manner.

- Thank you for catching this. We've edited to text to correct this.

9) Line 332: Bekinschtein references are missing in references list

- Thank you for catching this. We've corrected this.

Reviewer #4:

Manuscript Summary:

This manuscript details details an experimental protocol to investigate the neuronal mechanisms of short and long term olfactory learning and memory in rodents. While the protocol for these experiments was adequately described in the original manuscript by Tong, Kim and Cleland in Learning & Memory, 2018, 25: 198-205, a video protocol will be useful to the olfactory learning and memory research community.

- Thank you for this summary and for your review.

Major Concerns:

None.

Minor Concerns:

There are a couple of minor things that should be addressed before final publication, but for the most part the manuscript was well written and accurate.

1. The title is exactly the same as the original research article published in Learning & Memory, "Kinase activity in the olfactory bulb is required for odor memory consolidation" by Tong, Kim and Cleland in 2018. The title should reflect the protocol being described, not the results of the experiments that are already published elsewhere.

- Thank you for this. We agree with this and have changed the manuscript title to: “A low-cost, odor-reward association task for tests of learning and memory”

2. In section 1. Olfactory bulb calculation, the protocol goes straight from anesthesia (1.1) to guide cannula placement (1.2) without description of scalp deflection or drilling bur holes over the olfactory bulbs for cannula placement.

- Thank you for pointing this out. We added a great deal of description to this section, detailing the incision to the cement.

3. In section 2.1 Infusions, it is unclear what injection system was used for the micro infusions. It just says "Deliver infusions...", please include more detail.

- Thank you for this. We added the following sentences to clarify, the exact model will be available on the Table of Materials that accompanies the paper: “Infusions were delivered using a dual injector pump.”

4. The references are inconsistently formatted. In some the volume is in bold and others it isn't. Some have the first letter of each word in the article title capitalized and others only the first word is capitalized. So, while there are not big proofreading/editing problems, some attention does need to be paid to the reference section.

- Thank you. We have proofread this further.

Gmail Michelle Tong <michelletytong@gmail.com>

Re: Permission request - L&M, 2018, Author

3 messages

Mazzullo, Mala <mazzullo@cshl.edu> 30 January 2019 at 13:19
To: "tongmi@earlham.edu" <tongmi@earlham.edu>

Dear Michelle,

Thank you for submitting your permission request. Please note that authors of articles published in Learning & Memory retain copyright to their articles, as noted in your paper:

© 2018 Tong et al.; Published by Cold Spring Harbor Laboratory Press<http://www.learnmem.org/site/misc/terms.xhtml> This article is distributed exclusively by Cold Spring Harbor Laboratory Press for the first 12 months after the full-issue publication date (see http://learnmem.cshlp.org/site/misc/terms.xhtml). After 12 months, it is available under a Creative Commons License (Attribution-NonCommercial 4.0 International), as described at http://creativecommons.org/licenses/by-nc/4.0/.

Thus, you are free to reuse your figures in your work, provided that Learning & Memory is fully credited as the place of original publication and any modifications are noted. Additional terms and conditions of use may be found at http://learnmem.cshlp.org/site/misc/terms.xhtml.
Please don't hesitate to let me know if you have any additional questions or concerns.

Kind regards,
Mala

Mala Shwe Mazzullo
Executive Assistant to the Publisher
Cold Spring Harbor Laboratory Press
One Bungtown Road, Cold Spring Harbor, NY 11724
Tel: 516.422.4005 / www.cshlpress.org

From: "Macciola, Dana" <macciol@cshl.edu>
Date: Wednesday, January 30, 2019 at 10:59 AM
To: Mala Mazzullo <mazzullo@cshl.edu>
Subject: Permission request-L&M

Hi Mala,

Below is a permission request from Michelle Tong (first and corresponding author) for the manuscript below:

LEARNMEM/2017/046615

Kinase activity in the olfactory bulb is required for odor memory consolidation

Michelle T Tong

Let me know if you need any additional information.

Best,

Dana

On 1/30/19, 10:08 AM, "Michelle T Tong" <tongmi@earlham.edu> wrote:

Comments sent via LEARN. MEM. Feedback Page

TO: learnmem-feedback@highwire.stanford.edu

NAME: Michelle T Tong

EMAIL: tongmi@earlham.edu

IP ADDRESSES: 73.102.221.115, 73.102.221.115

HOSTNAME: c-73-102-221-115.hsd1.in.comcast.net

PREVIOUS PAGE: https://urldefense.proofpoint.com/v2/url?u=http-3A__learnmem.cshlp.org_help_&d=DwIBAg&c=mkpgQs82XaCKIwNV8b32dmVOmERqJe4bBOtF0CetP9Y&r=ze8TLxDzQZmCNsFev4BEZQ&m=A7e25bPirTU4O0-IUiICQKYkQELdz8ghpcGbcs_v6h8&s=3bk9e4kH6U75Hh_fL3_ykAo9xDiOPp980QR5qctW3gs&e=

BROWSER: Mozilla/5.0 (Macintosh; Intel Mac OS X 10_11_6) AppleWebKit/537.36 (KHTML, like Gecko) Chrome/71.0.3578.98 Safari/537.36, Learn. Mem.

PROMOTIONAL USE: Granted

SESSION ID: O5gEQXcgryv6aMz40N9sFg

COMMENTS:

Dear Learning & Memory,

I'm writing a methods paper for JoVE based on the methods in my Learning & Memory paper (Kinase activity in the olfactory bulb is required for odor memory consolidation, https://urldefense.proofpoint.com/v2/url?u=http-3A__www.learnmem.org_cgi_doi_10.1101_lm.046615&d=DwIBAg&c=mkpgQs82XaCKIwNV8b32dmVOmERqJe4bBOtF0CetP9Y&r=ze8TLxDzQZmCNsFev4BEZQ&m=A7e25bPirTU4O0-IUiICQKYkQELdz8ghpcGbcs_v6h8&s=b67V_teUaVYrdfSJhJ9U54WzlqC-xghXn0yIIEQwFLI&e=. 117) and I'm seeking permission for the reprint of Figure 1 and 2. Please advise.

Thanks so much,

Michelle Tong

Michelle Tong <tongmi@earlham.edu> 30 January 2019 at 14:41
To: "Mazzullo, Mala" <mazzullo@cshl.edu>

Dear Mala,

Thanks so much for the swift response, and for pointing me to the information.

Thanks kindly,

michelle.

Michelle Tong, PhD
Assistant Professor of Psychology/Neuroscience

Landrum Bolling Center 303
Earlham College
Richmond, IN 47374
US: +001 765 983 1764

[Quoted text hidden]

Mazzullo, Mala <mazzullo@cshl.edu> 30 January 2019 at 15:07
To: Michelle Tong <tongmi@earlham.edu>

My pleasure, Michelle!

Best,
Mala

Mala Shwe Mazzullo
Executive Assistant to the Publisher
Cold Spring Harbor Laboratory Press
One Bungtown Road, Cold Spring Harbor, NY 11724
Tel: 516.422.4005 / www.cshlpress.org

From: Michelle Tong <tongmi@earlham.edu>
Date: Wednesday, January 30, 2019 at 2:41 PM
To: Mala Mazzullo <mazzullo@cshl.edu>
Subject: Re: Permission request - L&M, 2018, Author

Dear Mala,

Thanks so much for the swift response, and for pointing me to the information.

Thanks kindly,

michelle.

Michelle Tong, PhD
Assistant Professor of Psychology/Neuroscience

Landrum Bolling Center 303
Earlham College
Richmond, IN 47374
US: +001 765 983 1764

On Wed, 30 Jan 2019 at 13:21, Mazzullo, Mala <mazzullo@cshl.edu<mailto:mazzullo@cshl.edu>> wrote:
Dear Michelle,

Thank you for submitting your permission request. Please note that authors of articles published in Learning & Memory retain copyright to their articles, as noted in your paper:

Please don't hesitate to let me know if you have any additional questions or concerns.

Kind regards,
Mala

Mala Shwe Mazzullo
Executive Assistant to the Publisher
Cold Spring Harbor Laboratory Press
One Bungtown Road, Cold Spring Harbor, NY 11724
Tel: 516.422.4005 / www.cshlpress.org<http://www.cshlpress.org>

From: "Macciola, Dana" <macciol@cshl.edu<mailto:macciol@cshl.edu>>
Date: Wednesday, January 30, 2019 at 10:59 AM
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Subject: Permission request-L&M

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LEARNMEM/2017/046615

Kinase activity in the olfactory bulb is required for odor memory consolidation

Michelle T Tong

Let me know if you need any additional information.

Best,

Dana

On 1/30/19, 10:08 AM, "Michelle T Tong" <tongmi@earlham.edu<mailto:tongmi@earlham.edu>> wrote:

Comments sent via LEARN. MEM. Feedback Page

TO: learnmem-feedback@highwire.stanford.edu<mailto:learnmem-feedback@highwire.stanford.edu>

NAME: Michelle T Tong

EMAIL: tongmi@earlham.edu<mailto:tongmi@earlham.edu>

IP ADDRESSES: 73.102.221.115, 73.102.221.115

HOSTNAME: c-73-102-221-115.hsd1.in.comcast.net<https://urldefense.proofpoint.com/v2/url?u=http-3A__c-2D73-2D102-2D221-2D115.hsd1.in.comcast.net&d=DwMFaQ&c=mkpgQs82XaCKIwNV8b32dmVOmERqJe4bBOtF0CetP9Y&r=nJlxw2bUsw5YIj9S9LTu-XPHf3xS3Ip_W-VfRAYLIXE&m=S2WvhH0tAZX84c7hjF29D5MTMf-8WoPYPlr5-SIYyPY&s=LrZKYMrt_lwrqBGkFmnAfF_9NLmPbNg6Bo6lwGivbCE&e=>

[Quoted text hidden]

Syntax is given for SPSS 22.0

TO CREATE THE LOGIT TRANSFORMED VARIABLE

Recall that the dependent measure, proportion correct, is *not* continuous and is *not* unbound. That is, the proportion correct data are not continuous for a given trial block (TB; only the values 0, 0.2, 0.4, 0.6, 0.8, and 1 are possible).

Thus, we need to perform a Logit transform on the dependent measure (http://www.stata.com/support/faqs/statistics/logit-transformation/) before doing a mixed model analysis.

(1) TB1 values of 0 were replaced with 0.01 and values of 1 were replaced with 0.99, else preserved to create TransTB1. This method is preferred to removal of 1's and 0's a general linear transformation because it preserves the shape of the data after the logit transform (see http://stats.stackexchange.com/questions/109702/empirical-logit-transformation-on-percentage-data/110037#110037).

(2) Then used the Compute variable function to logit transform (http://www.stata.com/support/faqs/statistics/logit-transformation/) TransTB1 to create "LogitTB1"

Sample syntax below for (1) and (2) below:

```
RECODE TB1 (0=0.01) (1=0.99) (ELSE=Copy) INTO TransTB1.
EXECUTE.

COMPUTE LogitTB1=LN(TransTB1/(1-TransTB1)).
EXECUTE.
```

The same was done for all the other Trial Blocks as well.

PERFORMING A LINEAR MIXED EFFECTS ANALYSIS

First, to ascertain that DRUG and VEHICLE groups are indeed *not* different during Training (i.e. initial learning)

We first create a data file where the trial blocks are structured in long form. We then need to run a mixed model below.

MouseID and OdourSet are the random effects. LogitTBs is the dependent measure, DrugGroup (K252a or vehicle) and PropCorrectTBs (Trial Blocks of Training) are the two factors.

The Syntax below was used:

```
DATASET ACTIVATE DataSet4.
MIXED LogitTBs BY DrugGroup PropCorrectTBs MouseID OdourSet
 /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0, ABSOLUTE) LCONVERGE(0,
ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
 /FIXED= DrugGroup PropCorrectTBs DrugGroup*PropCorrectTBs| SSTYPE(3)
 /METHOD=REML
 /PRINT=SOLUTION
 /RANDOM=intercept | subject(MouseID*OdourSet)
 /SAVE=FIXPRED PRED RESID
 /EMMEANS=TABLES(DrugGroup*PropCorrectTBs) COMPARE (DrugGroup)
ADJ(BONFERRONI)
 /EMMEANS=TABLES(DrugGroup*PropCorrectTBs) COMPARE
(PropCorrectTBs) ADJ(SIDAK).
```

Second, the best comparison to test the differential memory of the two groups during short-term and long-term memory is to show that TB1 for the K252a group at LTM is the only group that is significantly different from TRAINING TB4, and it is significantly differently from Vehicle TB1 at LTM.

The best way to do this is the do a linear mixed model. LogTrans is the logit transformed dependent measure, proportion correct. The factor Test_Training indicates whether or not the data are from the Training session or Testing Session. Short_Long indicates whether or not the mouse was in the STM group or the LTM group. DrugGroup (K252a or vehicle) indicates whether or not the mouse was in the K252a or vehicle group. MouseID nested in Short_Long and then nested in OdourSet are the random effects.

The Syntax below was used:

```
DATASET ACTIVATE DataSet2.
MIXED LogTrans BY Test_Training DrugGroup MouseID Short_Long OdourSet
 /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0, ABSOLUTE) LCONVERGE(0,
ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
 /FIXED=Test_Training DrugGroup Test_Training*DrugGroup Short_Long
Short_Long*Test_Training Short_Long*DrugGroup
Short_Long*DrugGroup*Test_Training| SSTYPE(3)
 /METHOD=REML
 /PRINT=SOLUTION
 /RANDOM=intercept | subject(MouseID*Short_Long)
 /RANDOM=intercept | subject(OdourSet*MouseID*Short_Long)
 /SAVE=FIXPRED PRED RESID
 /EMMEANS=TABLES(Test_Training*DrugGroup*Short_Long) COMPARE
(Short_Long) ADJ(SIDAK)
```

```
 /EMMEANS=TABLES(Test_Training*DrugGroup*Short_Long) COMPARE
(DrugGroup) ADJ(SIDAK)
/EMMEANS=TABLES(Test_Training*DrugGroup*Short_Long) COMPARE
(Test_Training) ADJ(BONFERRONI).
```

Analysis results found in: Discrim_Output_FULL.spv

Full model:

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	10.891	152.112	.000
Test_Training	1	60.916	.419	.520
DrugGroup	1	45.592	.838	.365
Test_Training * DrugGroup	1	60.916	.885	.351
Short_Long	1	10.891	.300	.595
Test_Training * Short_Long	1	60.916	4.423	.040
DrugGroup * Short_Long	1	45.592	2.100	.154
Test_Training * DrugGroup * Short_Long	1	60.916	5.025	.029

a. Dependent Variable: LogTBs.

Results: The three-way interaction and 1 two-way interactions are significant.

Proceed to the post-hocs:

Pairwise Comparisons[a]

Test_Training	DrugGroup	(I) Short_Long	(J) Short_Long	Mean Difference (I-J)	Std. Error	df	Sig.[c]
Training	Vehicle	STM	LTM	-.753	.748	68.733	.317
		LTM	STM	.753	.748	68.733	.317
	K252a	STM	LTM	-1.037	.717	59.472	.153
		LTM	STM	1.037	.717	59.472	.153
Test	Vehicle	STM	LTM	-.838	.752	70.683	.269
		LTM	STM	.838	.752	70.683	.269
	K252a	STM	LTM	1.632*	.717	59.472	.027
		LTM	STM	-1.632*	.717	59.472	.027

For the drug group, there is a significant difference between TB1 between STM and LTM (p = .027)

Importantly, no significant differences in training.

Pairwise Comparisons[a]

Test_Training	Short_Long	(I) DrugGroup	(J) DrugGroup	Mean Difference (I-J)	Std. Error	df	Sig.[c]
Training	STM	Vehicle	K252a	.198	.674	76.750	.769
		K252a	Vehicle	-.198	.674	76.750	.769
	LTM	Vehicle	K252a	-.086	.695	98.964	.902
		K252a	Vehicle	.086	.695	98.964	.902
Test	STM	Vehicle	K252a	-.601	.682	71.642	.381
		K252a	Vehicle	.601	.682	71.642	.381
	LTM	Vehicle	K252a	1.869*	.701	95.042	.009
		K252a	Vehicle	-1.869*	.701	95.042	.009

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogTBs.

c. Adjustment for multiple comparisons: Bonferroni.

The important comparison for us here is that for LTM, drug and vehicle group were different (p = .009)

Again, no significant differences in training.

Pairwise Comparisons[a]

DrugGroup	Short_Long	(I) Test_Training	(J) Test_Training	Mean Difference (I-J)	Std. Error	df	Sig.[c]
Vehicle	STM	Training	Test	-.048	.610	61.825	.938
		Test	Training	.048	.610	61.825	.938
	LTM	Training	Test	-.133	.648	59.407	.839
		Test	Training	.133	.648	59.407	.839
K252a	STM	Training	Test	-.847	.602	56.297	.165
		Test	Training	.847	.602	56.297	.165
	LTM	Training	Test	1.822*	.596	66.642	.003
		Test	Training	-1.822*	.596	66.642	.003

Based on estimated marginal means

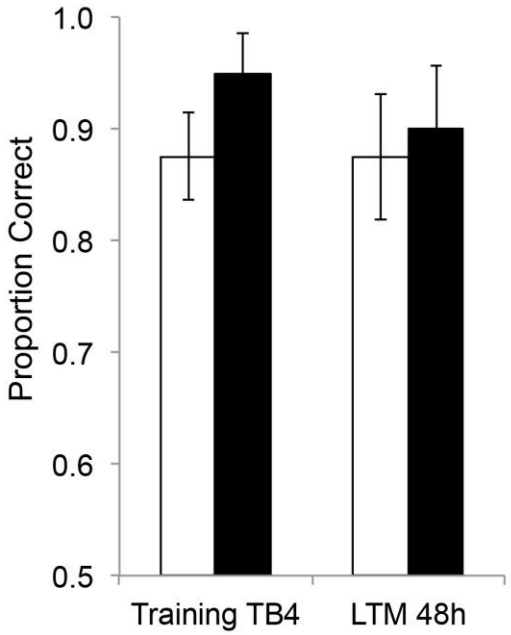
*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogTBs.

c. Adjustment for multiple comparisons: Bonferroni.

The only significant difference in this analysis is that for the drug group, in LTM the test and training measures were different. That is, TB1 for drug at LTM was significantly lower than TB4 at training (p = .003).

TO RUN THIS MIXED MODEL FOR THE RETRIEVAL CONTROL



We'll run a separate mixed model on the pre-retrieval infusion group, since these data were collected completely separately. In addition, it doesn't make sense to compare these with the other data because the infusion timing is different as well.

So using a new data file, found in: TONG_Discrim_Retrieval.sav

I created a new variable LogTBs. In this variable, for the TRAINING group LogitTB4 was copied, for any testing group, LogitTB1 was used.

This mixed model has two fixed effects: test_training and experimental group (which infusion they received). We do *not* have "short_long" since all animals are tested at LTM.

```
DATASET ACTIVATE DataSet1.
MIXED LogTBs BY Test_Training DrugGroup MouseID Trial
 /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0, ABSOLUTE) LCONVERGE(0,
ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
 /FIXED=Test_Training DrugGroup Test_Training*DrugGroup | SSTYPE(3)
 /METHOD=REML
 /PRINT=SOLUTION
 /RANDOM=intercept | subject(Trial*MouseID)
 /SAVE=FIXPRED PRED RESID
 /EMMEANS=TABLES(Test_Training*DrugGroup) COMPARE (DrugGroup)
ADJ(BONFERRONI)
 /EMMEANS=TABLES(Test_Training*DrugGroup) COMPARE (Test_Training)
ADJ(BONFERRONI).
```

Full Model:

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	14	82.855	.000
Test_Training	1	14	.055	.817
DrugGroup	1	14	1.361	.263
Test_Training * DrugGroup	1	14	.592	.454

a. Dependent Variable: LogTBs.

No significant interaction or main effects.

Post-hocs show no significance for all pairwise comparisons:

Pairwise Comparisons[a]

Test_Training	(I) DrugGroup	(J) DrugGroup	Mean Difference (I-J)	Std. Error	df	Sig.[b]
Training	Vehicle	K252a	1.203	.863	26.097	.175
	K252a	Vehicle	-1.203	.863	26.097	.175
TestRetr	Vehicle	K252a	.401	.863	26.097	.646
	K252a	Vehicle	-.401	.863	26.097	.646

Based on estimated marginal means

a. Dependent Variable: LogTBs.

b. Adjustment for multiple comparisons: Bonferroni.

Pairwise Comparisons[a]

DrugGroup	(I) Test_Training	(J) Test_Training	Mean Difference (I-J)	Std. Error	df	Sig.[b]
Vehicle	Training	TestRetr	.524	.737	14	.489
	TestRetr	Training	-.524	.737	14	.489
K252a	Training	TestRetr	-.278	.737	14	.711
	TestRetr	Training	.278	.737	14	.711

Based on estimated marginal means

a. Dependent Variable: LogTBs.

b. Adjustment for multiple comparisons: Bonferroni.

TO ANALYZE THE DISCRIMINATION INDEX DATA

To investigate the relationships between the Discrimination Index.

We want to show that for LTM at TESTING the K252a group has a lower Discrimination Index than the others.

To do this, we convert the original data set to a long form, where a new variable, called DigTrial, will indicate T1, T5, T10, T15, T20. It's easier to use the Data -> Restructure function so you can sort of visualize what you want as you work,b ut the syntax is below as well:

```
VARSTOCASES
  /MAKE DigTrial FROM LogitDiscrimIndeT1 LogitDiscrimIndeT5
LogitDiscrimIndeT10 LogitDiscrimIndeT15
    LogitDiscrimIndeT20
  /INDEX=TrialPeriod(DigTrial)
  /KEEP=MouseID Trial Test_Training Short_Long DrugGroup LogitTB1 LogitTB2
LogitTB3 LogitTB4 DigT1R
    DigT1U DigT5R DigT5U DigT10R DigT10U DigT15R DigT15U DigT20R
DigT20U T1 T2 T3 T4 T5 T6 T7 T8 T9 T10
    T11 T12 T13 T14 T15 T16 T17 T18 T19 T20 TB1 TB2 TB3 TB4
DiscrimIndeT1 DiscrimIndeT5 DiscrimIndeT10
```

```
    DiscrimIndeT15 DiscrimIndeT20 TransDiscrimIndeT1 TransDiscrimIndeT5
TransDiscrimIndeT10
    TransDiscrimIndeT15 TransDiscrimIndeT20 TransTB1 TransTB2 TransTB3
TransTB4
  /NULL=KEEP.
```

Then we can apply the syntax below to make the fixed effects comparison. We used a data file with just the STM/LTM data (no Retrieval):

```
DATASET ACTIVATE DataSet3.
MIXED LogDiscrim BY Test_Training DrugGroup TrialPeriod Short_Long Trial
MouseID
 /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0, ABSOLUTE) LCONVERGE(0,
ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
/FIXED=Test_Training Short_Long DrugGroup TrialPeriod
Test_Training*Short_Long
    Test_Training*DrugGroup Test_Training*TrialPeriod Short_Long*DrugGroup
Short_Long*TrialPeriod
    DrugGroup*TrialPeriod Test_Training*Short_Long*DrugGroup
Test_Training*Short_Long*TrialPeriod
    Test_Training*DrugGroup*TrialPeriod Short_Long*DrugGroup*TrialPeriod
Test_Training*Short_Long*DrugGroup*TrialPeriod | SSTYPE(3)
 /METHOD=REML
 /PRINT=SOLUTION
 /RANDOM=intercept | subject(MouseID)
 /RANDOM=intercept | subject(Trial*MouseID)
 /SAVE=FIXPRED PRED RESID
 /EMMEANS=TABLES(Short_Long*DrugGroup*Test_Training*TrialPeriod)
COMPARE (Short_Long) ADJ(BONFERRONI)
 /EMMEANS=TABLES(Short_Long*DrugGroup*Test_Training*TrialPeriod)
COMPARE (DrugGroup) ADJ(BONFERRONI)
 /EMMEANS=TABLES(Short_Long*DrugGroup*Test_Training*TrialPeriod)
COMPARE (Test_Training) ADJ(BONFERRONI)
/EMMEANS=TABLES(Short_Long*DrugGroup*Test_Training*TrialPeriod)
COMPARE (TrialPeriod) ADJ(BONFERRONI).
```

The full model yields the following Fixed Effects table:

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	15.427	118.918	.000
Test_Training	1	500.794	75.213	.000
Short_Long	1	15.427	2.067	.170
DrugGroup	1	50.864	2.257	.139
TrialPeriod	4	466.497	42.601	.000
Test_Training * Short_Long	1	500.794	.262	.609
Test_Training * DrugGroup	1	500.695	1.263	.262
Test_Training * TrialPeriod	4	466.975	16.908	.000
DrugGroup * Short_Long	1	50.864	1.255	.268
TrialPeriod * Short_Long	4	466.497	1.221	.301
DrugGroup * TrialPeriod	4	466.331	.664	.617
Test_Training * DrugGroup * Short_Long	1	500.695	1.229	.268
Test_Training * TrialPeriod * Short_Long	4	466.975	2.278	.060
Test_Training * DrugGroup * TrialPeriod	4	466.864	1.268	.282
DrugGroup * TrialPeriod * Short_Long	4	466.331	1.271	.281
Test_Training * DrugGroup * TrialPeriod * Short_Long	4	466.864	3.036	.017

a. Dependent Variable: LogDiscrim.

From this, we see that we do have a significant 4-way interaction ($p < .05$). A significant two-way interaction of Testing_Training and TrialPeriod ($p < .05$). Finally, we find significant main effects of Test_Training ($p < .05$) and TrialPeriod

($p < .05$), meaning that DIs differ between the Testing and Training phases, and that DIs differ between T1-T20.
We can see where exactly these are in the post-hoc comparisons.

In the post-hoc comparisons (Bonferroni correction for multiple comparisons):

Pairwise Comparisons[a]

DrugGroup	Test_Training	TrialPeriod	(I) Short_Long	(J) Short_Long	Mean Difference (I-J)	Std. Error	df	Sig.[c]
Vehicle	Training	LogitDiscrimInde T1	STM	LTM	-2.580*	1.171	470.184	.028
			LTM	STM	2.580*	1.171	470.184	.028
		LogitDiscrimInde T10	STM	LTM	.125	.797	287.284	.876
			LTM	STM	-.125	.797	287.284	.876
		LogitDiscrimInde T15	STM	LTM	-1.190	.804	289.502	.140
			LTM	STM	1.190	.804	289.502	.140
		LogitDiscrimInde T20	STM	LTM	-1.681*	.788	278.984	.034
			LTM	STM	1.681*	.788	278.984	.034
		LogitDiscrimInde T5	STM	LTM	1.146	.814	297.190	.160
			LTM	STM	-1.146	.814	297.190	.160
	Test	LogitDiscrimInde T1	STM	LTM	-1.136	.789	280.021	.151
			LTM	STM	1.136	.789	280.021	.151
		LogitDiscrimInde T10	STM	LTM	-.787	.775	269.767	.311
			LTM	STM	.787	.775	269.767	.311
		LogitDiscrimInde T15	STM	LTM	.208	.775	269.767	.788
			LTM	STM	-.208	.775	269.767	.788
		LogitDiscrimInde T20	STM	LTM	.101	.789	279.020	.898
			LTM	STM	-.101	.789	279.020	.898
		LogitDiscrimInde T5	STM	LTM	-1.564*	.789	279.020	.048
			LTM	STM	1.564*	.789	279.020	.048
K252a	Training	LogitDiscrimInde T1	STM	LTM	-.712	.941	383.106	.449
			LTM	STM	.712	.941	383.106	.449
		LogitDiscrimInde T10	STM	LTM	-.176	.752	246.976	.815
			LTM	STM	.176	.752	246.976	.815
		LogitDiscrimInde T15	STM	LTM	.544	.752	249.654	.470
			LTM	STM	-.544	.752	249.654	.470
		LogitDiscrimInde T20	STM	LTM	-.185	.751	247.448	.805
			LTM	STM	.185	.751	247.448	.805
		LogitDiscrimInde T5	STM	LTM	.622	.786	270.777	.429
			LTM	STM	-.622	.786	270.777	.429
	Test	LogitDiscrimInde T1	STM	LTM	.702	.815	292.687	.389
			LTM	STM	-.702	.815	292.687	.389
		LogitDiscrimInde T10	STM	LTM	-1.273	.752	246.890	.092
			LTM	STM	1.273	.752	246.890	.092
		LogitDiscrimInde T15	STM	LTM	-1.927*	.790	271.384	.015
			LTM	STM	1.927*	.790	271.384	.015
		LogitDiscrimInde T20	STM	LTM	-.700	.763	254.793	.359
			LTM	STM	.700	.763	254.793	.359
		LogitDiscrimInde T5	STM	LTM	.566	.763	254.279	.459
			LTM	STM	-.566	.763	254.279	.459

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogDiscrim.

c. Adjustment for multiple comparisons: Bonferroni.

I don't think these comparisons tell us anything too too interesting.

NEXT

Pairwise Comparisons[a]

Short_Long	Test_Training	TrialPeriod	(I) DrugGroup	(J) DrugGroup	Mean Difference (I-J)	Std. Error	df	Sig.[c]
STM	Training	LogitDiscrimInde T1	Vehicle	K252a	-1.153	.986	502.518	.243
			K252a	Vehicle	1.153	.986	502.518	.243
		LogitDiscrimInde T10	Vehicle	K252a	-.802	.708	469.118	.258
			K252a	Vehicle	.802	.708	469.118	.258
		LogitDiscrimInde T15	Vehicle	K252a	-.584	.708	468.725	.410
			K252a	Vehicle	.584	.708	468.725	.410
		LogitDiscrimInde T20	Vehicle	K252a	-1.513*	.697	465.149	.030
			K252a	Vehicle	1.513*	.697	465.149	.030
		LogitDiscrimInde T5	Vehicle	K252a	-.646	.719	472.300	.370
			K252a	Vehicle	.646	.719	472.300	.370
	Test	LogitDiscrimInde T1	Vehicle	K252a	-.357	.711	453.856	.616
			K252a	Vehicle	.357	.711	453.856	.616
		LogitDiscrimInde T10	Vehicle	K252a	.148	.700	449.454	.832
			K252a	Vehicle	-.148	.700	449.454	.832
		LogitDiscrimInde T15	Vehicle	K252a	.393	.700	449.454	.574
			K252a	Vehicle	-.393	.700	449.454	.574
		LogitDiscrimInde T20	Vehicle	K252a	-.151	.700	449.454	.829
			K252a	Vehicle	.151	.700	449.454	.829
		LogitDiscrimInde T5	Vehicle	K252a	-.978	.700	449.454	.163
			K252a	Vehicle	.978	.700	449.454	.163
LTM	Training	LogitDiscrimInde T1	Vehicle	K252a	.715	1.068	510.992	.504
			K252a	Vehicle	-.715	1.068	510.992	.504
		LogitDiscrimInde T10	Vehicle	K252a	-1.103	.743	495.359	.138
			K252a	Vehicle	1.103	.743	495.359	.138
		LogitDiscrimInde T15	Vehicle	K252a	1.150	.751	474.982	.126
			K252a	Vehicle	-1.150	.751	474.982	.126
		LogitDiscrimInde T20	Vehicle	K252a	-.016	.745	480.333	.982
			K252a	Vehicle	.016	.745	480.333	.982
		LogitDiscrimInde T5	Vehicle	K252a	-1.169	.785	485.443	.137
			K252a	Vehicle	1.169	.785	485.443	.137
	Test	LogitDiscrimInde T1	Vehicle	K252a	1.482	.798	488.390	.064
			K252a	Vehicle	-1.482	.798	488.390	.064
		LogitDiscrimInde T10	Vehicle	K252a	-.338	.731	474.334	.644
			K252a	Vehicle	.338	.731	474.334	.644
		LogitDiscrimInde T15	Vehicle	K252a	-1.743*	.769	486.240	.024
			K252a	Vehicle	1.743*	.769	486.240	.024
		LogitDiscrimInde T20	Vehicle	K252a	-.952	.756	478.916	.208
			K252a	Vehicle	.952	.756	478.916	.208
		LogitDiscrimInde T5	Vehicle	K252a	1.152	.756	478.632	.128
			K252a	Vehicle	-1.152	.756	478.632	.128

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogDiscrim.

c. Adjustment for multiple comparisons: Bonferroni.

In these comparisons, we see that during the LTM test period, the DI on T1 is marginally different between the vehicle and drug group (p = .064). Importantly, the same comparison for STM is not significantly different (p > .05).

NEXT:

Pairwise Comparisons[a]

Short_Long	DrugGroup	TrialPeriod	(I) Test_Training	(J) Test_Training	Mean Difference (I-J)	Std. Error	df	Sig.[c]
STM	Vehicle	LogitDiscrimInde T1	Training	Test	-4.867*	.930	491.215	.000
			Test	Training	4.867*	.930	491.215	.000
		LogitDiscrimInde T10	Training	Test	-1.116	.689	472.031	.106
			Test	Training	1.116	.689	472.031	.106
		LogitDiscrimInde T15	Training	Test	-.759	.677	469.777	.263
			Test	Training	.759	.677	469.777	.263
		LogitDiscrimInde T20	Training	Test	-.580	.677	469.777	.392
			Test	Training	.580	.677	469.777	.392
		LogitDiscrimInde T5	Training	Test	-1.415*	.689	471.766	.040
			Test	Training	1.415*	.689	471.766	.040
	K252a	LogitDiscrimInde T1	Training	Test	-4.070*	.742	471.265	.000
			Test	Training	4.070*	.742	471.265	.000
		LogitDiscrimInde T10	Training	Test	-.165	.675	460.411	.807
			Test	Training	.165	.675	460.411	.807
		LogitDiscrimInde T15	Training	Test	.218	.686	462.184	.751
			Test	Training	-.218	.686	462.184	.751
		LogitDiscrimInde T20	Training	Test	.782	.675	460.411	.247
			Test	Training	-.782	.675	460.411	.247
		LogitDiscrimInde T5	Training	Test	-1.747*	.686	462.396	.011
			Test	Training	1.747*	.686	462.396	.011
LTM	Vehicle	LogitDiscrimInde T1	Training	Test	-3.423*	.956	485.009	.000
			Test	Training	3.423*	.956	485.009	.000
		LogitDiscrimInde T10	Training	Test	-2.027*	.737	468.391	.006
			Test	Training	2.027*	.737	468.391	.006
		LogitDiscrimInde T15	Training	Test	.640	.753	466.747	.396
			Test	Training	-.640	.753	466.747	.396
		LogitDiscrimInde T20	Training	Test	1.203	.751	467.078	.110
			Test	Training	-1.203	.751	467.078	.110
		LogitDiscrimInde T5	Training	Test	-4.125*	.765	463.859	.000
			Test	Training	4.125*	.765	463.859	.000
	K252a	LogitDiscrimInde T1	Training	Test	-2.656*	.892	504.257	.003
			Test	Training	2.656*	.892	504.257	.003
		LogitDiscrimInde T10	Training	Test	-1.263	.678	478.698	.063
			Test	Training	1.263	.678	478.698	.063
		LogitDiscrimInde T15	Training	Test	-2.253*	.711	489.307	.002
			Test	Training	2.253*	.711	489.307	.002
		LogitDiscrimInde T20	Training	Test	.267	.691	481.816	.699
			Test	Training	-.267	.691	481.816	.699
		LogitDiscrimInde T5	Training	Test	-1.803*	.716	487.038	.012
			Test	Training	1.803*	.716	487.038	.012

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogDiscrim.

c. Adjustment for multiple comparisons: Bonferroni.

This comparison tells us that the DIs of T1 for Test at STM and LTM are significantly higher for than the DIs of Training. This makes sense. It confirms that learning occurred during training and that in all cases, even the LTM K252a group, there was some retention of the learning (at least in terms of confidence?)

NEXT:

Pairwise Comparisons[a]

Short_Long	DrugGroup	Test_Training	(I) TrialPeriod	(J) TrialPeriod	Mean Difference (I-J)	Std. Error	df	Sig.[c]
STM	Vehicle	Training	LogitDiscrimInde T1	LogitDiscrimInde T10	-4.372*	.934	481.448	.000
				LogitDiscrimInde T15	-5.344*	.926	482.624	.000
				LogitDiscrimInde T20	-5.560*	.926	482.624	.000
				LogitDiscrimInde T5	-3.624*	.934	481.437	.001
			LogitDiscrimInde T10	LogitDiscrimInde T1	4.372*	.934	481.448	.000
				LogitDiscrimInde T15	-.972	.686	462.405	1.000
				LogitDiscrimInde T20	-1.188	.686	462.405	.841
				LogitDiscrimInde T5	.748	.698	464.452	1.000
			LogitDiscrimInde T15	LogitDiscrimInde T1	5.344*	.926	482.624	.000
				LogitDiscrimInde T10	.972	.686	462.405	1.000
				LogitDiscrimInde T20	-.216	.675	460.411	1.000
				LogitDiscrimInde T5	1.720	.686	462.388	.126
			LogitDiscrimInde T20	LogitDiscrimInde T1	5.560*	.926	482.624	.000
				LogitDiscrimInde T10	1.188	.686	462.405	.841
				LogitDiscrimInde T15	.216	.675	460.411	1.000
				LogitDiscrimInde T5	1.936	.686	462.388	.050
			LogitDiscrimInde T5	LogitDiscrimInde T1	3.624*	.934	481.437	.001
				LogitDiscrimInde T10	-.748	.698	464.452	1.000
				LogitDiscrimInde T15	-1.720	.686	462.388	.126
				LogitDiscrimInde T20	-1.936	.686	462.388	.050
		Test	LogitDiscrimInde T1	LogitDiscrimInde T10	-.621	.675	460.411	1.000
				LogitDiscrimInde T15	-1.235	.675	460.411	.677
				LogitDiscrimInde T20	-1.273	.675	460.411	.598
				LogitDiscrimInde T5	-.172	.675	460.411	1.000
			LogitDiscrimInde T10	LogitDiscrimInde T1	.621	.675	460.411	1.000
				LogitDiscrimInde T15	-.615	.675	460.411	1.000
				LogitDiscrimInde T20	-.652	.675	460.411	1.000
				LogitDiscrimInde T5	.448	.675	460.411	1.000
			LogitDiscrimInde T15	LogitDiscrimInde T1	1.235	.675	460.411	.677
				LogitDiscrimInde T10	.615	.675	460.411	1.000
				LogitDiscrimInde T20	-.037	.675	460.411	1.000
				LogitDiscrimInde T5	1.063	.675	460.411	1.000

		LogitDiscrimInde T5	LogitDiscrimInde T1	.172	.675	460.411	1.000
			LogitDiscrimInde T10	-.448	.675	460.411	1.000
			LogitDiscrimInde T15	-1.063	.675	460.411	1.000
			LogitDiscrimInde T20	-1.101	.675	460.411	1.000
K252a	Training	LogitDiscrimInde T1	LogitDiscrimInde T10	-4.021*	.731	468.542	.000
			LogitDiscrimInde T15	-4.775*	.743	470.633	.000
			LogitDiscrimInde T20	-5.920*	.731	468.542	.000
			LogitDiscrimInde T5	-3.117*	.741	466.587	.000
		LogitDiscrimInde T10	LogitDiscrimInde T1	4.021*	.731	468.542	.000
			LogitDiscrimInde T15	-.754	.686	462.184	1.000
			LogitDiscrimInde T20	-1.899	.675	460.411	.051
			LogitDiscrimInde T5	.904	.686	462.396	1.000
		LogitDiscrimInde T15	LogitDiscrimInde T1	4.775*	.743	470.633	.000
			LogitDiscrimInde T10	.754	.686	462.184	1.000
			LogitDiscrimInde T20	-1.145	.686	462.184	.960
			LogitDiscrimInde T5	1.658	.698	464.261	.180
		LogitDiscrimInde T15	LogitDiscrimInde T1	4.775*	.743	470.633	.000
			LogitDiscrimInde T10	.754	.686	462.184	1.000
			LogitDiscrimInde T20	-1.145	.686	462.184	.960
			LogitDiscrimInde T5	1.658	.698	464.261	.180
		LogitDiscrimInde T20	LogitDiscrimInde T1	5.920*	.731	468.542	.000
			LogitDiscrimInde T10	1.899	.675	460.411	.051
			LogitDiscrimInde T15	1.145	.686	462.184	.960
			LogitDiscrimInde T5	2.803*	.686	462.396	.001
		LogitDiscrimInde T5	LogitDiscrimInde T1	3.117*	.741	466.587	.000
			LogitDiscrimInde T10	-.904	.686	462.396	1.000
			LogitDiscrimInde T15	-1.658	.698	464.261	.180
			LogitDiscrimInde T20	-2.803*	.686	462.396	.001
	Test	LogitDiscrimInde T1	LogitDiscrimInde T10	-.116	.686	462.170	1.000
			LogitDiscrimInde T15	-.486	.686	462.170	1.000
			LogitDiscrimInde T20	-1.067	.686	462.170	1.000
			LogitDiscrimInde T5	-.794	.686	462.170	1.000

			LogitDiscrimInde T10	LogitDiscrimInde T1	.116	.686	462.170	1.000
				LogitDiscrimInde T15	-.370	.675	460.411	1.000
				LogitDiscrimInde T20	-.952	.675	460.411	1.000
				LogitDiscrimInde T5	-.678	.675	460.411	1.000
			LogitDiscrimInde T15	LogitDiscrimInde T1	.486	.686	462.170	1.000
				LogitDiscrimInde T10	.370	.675	460.411	1.000
				LogitDiscrimInde T20	-.582	.675	460.411	1.000
				LogitDiscrimInde T5	-.308	.675	460.411	1.000
			LogitDiscrimInde T20	LogitDiscrimInde T1	1.067	.686	462.170	1.000
				LogitDiscrimInde T10	.952	.675	460.411	1.000
				LogitDiscrimInde T15	.582	.675	460.411	1.000
				LogitDiscrimInde T5	.274	.675	460.411	1.000
			LogitDiscrimInde T5	LogitDiscrimInde T1	.794	.686	462.170	1.000
				LogitDiscrimInde T10	.678	.675	460.411	1.000
				LogitDiscrimInde T15	.308	.675	460.411	1.000
				LogitDiscrimInde T20	-.274	.675	460.411	1.000
LTM	Vehicle	Training	LogitDiscrimInde T1	LogitDiscrimInde T10	-1.667	.956	484.299	.817
				LogitDiscrimInde T15	-3.954*	.963	476.976	.000
				LogitDiscrimInde T20	-4.662*	.952	479.452	.000
				LogitDiscrimInde T5	.102	.963	476.976	1.000
			LogitDiscrimInde T10	LogitDiscrimInde T1	1.667	.956	484.299	.817
				LogitDiscrimInde T15	-2.287*	.767	468.111	.030
				LogitDiscrimInde T20	-2.995*	.750	464.604	.001
				LogitDiscrimInde T5	1.769	.767	468.111	.216
			LogitDiscrimInde T15	LogitDiscrimInde T1	3.954*	.963	476.976	.000
				LogitDiscrimInde T10	2.287*	.767	468.111	.030
				LogitDiscrimInde T20	-.708	.765	463.777	1.000
				LogitDiscrimInde T5	4.056*	.779	460.411	.000
			LogitDiscrimInde T20	LogitDiscrimInde T1	4.662*	.952	479.452	.000
				LogitDiscrimInde T10	2.995*	.750	464.604	.001
				LogitDiscrimInde T15	.708	.765	463.777	1.000
				LogitDiscrimInde T5	4.764*	.765	463.777	.000

		LogitDiscrimInde T5	LogitDiscrimInde T1	-.102	.963	476.976	1.000
			LogitDiscrimInde T10	-1.769	.767	468.111	.216
			LogitDiscrimInde T15	-4.056*	.779	460.411	.000
			LogitDiscrimInde T20	-4.764*	.765	463.777	.000
	Test	LogitDiscrimInde T1	LogitDiscrimInde T10	-.271	.736	462.291	1.000
			LogitDiscrimInde T15	.109	.736	462.291	1.000
			LogitDiscrimInde T20	-.035	.751	465.709	1.000
			LogitDiscrimInde T5	-.600	.751	465.709	1.000
		LogitDiscrimInde T10	LogitDiscrimInde T1	.271	.736	462.291	1.000
			LogitDiscrimInde T15	.381	.721	460.411	1.000
			LogitDiscrimInde T20	.236	.736	463.544	1.000
			LogitDiscrimInde T5	-.328	.736	463.544	1.000
		LogitDiscrimInde T15	LogitDiscrimInde T1	-.109	.736	462.291	1.000
			LogitDiscrimInde T10	-.381	.721	460.411	1.000
			LogitDiscrimInde T20	-.145	.736	463.544	1.000
			LogitDiscrimInde T5	-.709	.736	463.544	1.000
		LogitDiscrimInde T5	LogitDiscrimInde T1	.600	.751	465.709	1.000
			LogitDiscrimInde T10	.328	.736	463.544	1.000
			LogitDiscrimInde T15	.709	.736	463.544	1.000
			LogitDiscrimInde T20	.564	.748	460.411	1.000
K252a	Training	LogitDiscrimInde T1	LogitDiscrimInde T10	-3.484*	.836	481.069	.000
			LogitDiscrimInde T15	-3.518*	.829	483.628	.000
			LogitDiscrimInde T20	-5.393*	.836	481.635	.000
			LogitDiscrimInde T5	-1.782	.856	480.951	.380
		LogitDiscrimInde T10	LogitDiscrimInde T1	3.484*	.836	481.069	.000
			LogitDiscrimInde T15	-.034	.666	464.187	1.000
			LogitDiscrimInde T20	-1.908	.677	467.177	.050
			LogitDiscrimInde T5	1.702	.700	464.870	.154
		LogitDiscrimInde T15	LogitDiscrimInde T1	3.518*	.829	483.628	.000
			LogitDiscrimInde T10	.034	.666	464.187	1.000
			LogitDiscrimInde T20	-1.874	.666	463.077	.051
			LogitDiscrimInde T5	1.736	.691	468.343	.124

	LogitDiscrimInde T20	LogitDiscrimInde T1	5.393*	.836	481.635	.000
		LogitDiscrimInde T10	1.908	.677	467.177	.050
		LogitDiscrimInde T15	1.874	.666	463.077	.051
		LogitDiscrimInde T5	3.611*	.700	466.034	.000
	LogitDiscrimInde T5	LogitDiscrimInde T1	1.782		480.951	.380
		LogitDiscrimInde T10	-1.702	.700	464.87	.154
		LogitDiscrimInde T15	-1.736	.691	468.343	.12
		LogitDiscrimInde T20	-3.611*	.700	466.034	.000
Test	LogitDiscrimInde T1	LogitDiscrimInde T10	-2.091*	.732	469.206	.045
		LogitDiscrimInde T15	-3.115*	.767	468.278	.001
		LogitDiscrimInde T20	-2.470*	.744	474.146	.010
		LogitDiscrimInde T5	-.930	.742	466.599	1.000
	LogitDiscrimInde T10	LogitDiscrimInde T1	2.091*	.732	469.206	.045
		LogitDiscrimInde T15	-1.024	.715	467.610	1.000
		LogitDiscrimInde T20	-.379	.689	469.769	1.000
		LogitDiscrimInde T5	1.162	.687	462.885	.914
	LogitDiscrimInde T15	LogitDiscrimInde T1	3.115*	.767	468.278	.001
		LogitDiscrimInde T10	1.024	.715	467.610	1.000
		LogitDiscrimInde T20	.645	.725	466.552	1.000
		LogitDiscrimInde T5	2.186*	.725	465.263	.027
	LogitDiscrimInde T20	LogitDiscrimInde T1		.744	474.146	.010
		LogitDiscrimInde T10	.379	.689	469.769	1.000
		LogitDiscrimInde T15	-.645	.725	466.552	1.000
		LogitDiscrimInde T5	1.540	.699	467.618	.280
	LogitDiscrimInde T5	LogitDiscrimInde T1	.930	.742	466.599	1.000
		LogitDiscrimInde T10	-1.162	.687	462.885	.914
		LogitDiscrimInde T15	-2.186*	.725	465.263	.027
		LogitDiscrimInde T20	-1.540	.699	467.618	.280

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogDiscrim.

c. Adjustment for multiple comparisons: Bonferroni.

In this complicated set of comparisons, we see that for all groups during training, the DI at T1 is significantly different from T20 ($p < .05$). This confirms that all animals learn to discriminate the odours during training. The interesting results are that during the STM testing period, no trials are different from each other for either K252a ($p > .05$) and Vehicle ($p > .05$) groups. This suggests that no re-learning occurred during the testing phases for these groups. Perhaps the strength of association was already saturated via intact memory retention. During

the LTM testing period, the Vehicle group likewise shows no differences between any of the trials (*ps* > .05). In contrast, during LTM testing for the K252a (the one which showed decreased memory retention from the proportion correct data) we see that the DI at T20 is significantly higher than T1 ($p < .05$) suggesting that some learning is actually occurring during the testing trials, perhaps because of poor memory, associative strength still exists.

Since the previous analysis gave me a *p* = .064 for the key comparison, I'm curious it's because I'm not making the comparisons directly. To do this, I needed to recode the data as below:

Output: Discrim_Output_Index.spv
Data file: TONG_Discrim_LongFormWithNew.sav

I created the variable "New" that encompasses Test_Training and Short_Long, by having 4 levels.

- when Test_Training = Training, Short_Long = STM; New = 1 and combinations thereof for NEW= 2,3,4
- The purpose of this is to allow for the data points within these to be directly compared to one another, instead of the average of each label.

For the SYNTAX

```
DATASET ACTIVATE DataSet3.
MIXED LogDiscrim BY New DrugGroup TrialPeriod Trial MouseID
 /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0, ABSOLUTE) LCONVERGE(0,
ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
/FIXED=New DrugGroup TrialPeriod
    New*DrugGroup New*TrialPeriod
    DrugGroup*TrialPeriod New*DrugGroup*TrialPeriod | SSTYPE(3)
 /METHOD=REML
 /PRINT=SOLUTION
 /RANDOM=intercept | subject(MouseID)
 /RANDOM=intercept | subject(Trial*MouseID)
 /SAVE=FIXPRED PRED RESID
 /EMMEANS=TABLES(DrugGroup*New*TrialPeriod) COMPARE (New)
ADJ(BONFERRONI).
```

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	19.215	99.144	.000
New	3	75.183	30.072	.000
DrugGroup	1	58.968	3.475	.067
TrialPeriod	4	521.296	47.415	.000
New * DrugGroup	3	223.429	1.306	.273
New * TrialPeriod	12	521.414	7.235	.000
DrugGroup * TrialPeriod	4	521.128	.531	.713
New * DrugGroup * TrialPeriod	12	521.238	1.892	.033

a. Dependent Variable: LogDiscrim.

There is the significant 3-way interaction, but …

LTM-Test	LogitDiscrimInde T1	Vehicle	K252a	1.473	.787	565.755	.062
		K252a	Vehicle	-1.473	.787	565.755	.062
	LogitDiscrimInde T10	Vehicle	K252a	-.358	.721	555.691	.619
		K252a	Vehicle	.358	.721	555.691	.619
	LogitDiscrimInde T15	Vehicle	K252a	-1.749*	.759	565.133	.021
		K252a	Vehicle	1.749*	.759	565.133	.021
	LogitDiscrimInde T20	Vehicle	K252a	-.985	.745	558.716	.187
		K252a	Vehicle	.985	.745	558.716	.187
	LogitDiscrimInde T5	Vehicle	K252a	1.121	.745	558.637	.133
		K252a	Vehicle	-1.121	.745	558.637	.133

Here again, the key comparison has p = .062, marginal.

TO ANALYZE DIFFERENCES IN DISCRIMINATION INDEX GIVEN CORRECT OR INCORRECT T1

Data File: TONG_Discrim_LongFormDigYN_STM-LTM.sav

Here, our interest is in exploring any differences that exist between the infusion groups, testing groups depending on their correct or incorrect choice on each of T1, T5, T10, T15, T20. We'll remove the training trials from the data file and just look at STM and LTM.

To do this we run a mixed model with 4 fixed factors: infusion, STM/LTM, Correct/Incorrect (called T), and which trial (called TrialPeriod below)

```
DATASET ACTIVATE DataSet1.
MIXED LogitDiscrim BY DrugGroup Short_Long TrialPeriod T MouseID Trial
 /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0, ABSOLUTE) LCONVERGE(0,
ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
 /FIXED= DrugGroup Short_Long [TrialPeriod] [T]
[TrialPeriod]*DrugGroup [TrialPeriod]*Short_Long Short_Long*DrugGroup
[T]*DrugGroup [T]*Short_Long [T]*[TrialPeriod]
Short_Long*DrugGroup*[TrialPeriod] [T]*[TrialPeriod]*DrugGroup
[T]*[TrialPeriod]*Short_Long [T]*Short_Long*DrugGroup
DrugGroup*Short_Long*[TrialPeriod]*[T] | SSTYPE(3)
 /METHOD=REML
 /PRINT=SOLUTION
 /RANDOM=intercept | subject(MouseID*Short_Long)
 /RANDOM=intercept | subject(Trial*MouseID*Short_Long)
 /SAVE=FIXPRED PRED RESID
 /EMMEANS=TABLES(DrugGroup*Trial*Short_Long*[TrialPeriod]*[T])
COMPARE (DrugGroup) ADJ(BONFERRONI)
 /EMMEANS=TABLES(DrugGroup*Trial*Short_Long*[TrialPeriod]*[T])
COMPARE ([TrialPeriod]) ADJ(BONFERRONI)
 /EMMEANS=TABLES(DrugGroup*Trial*Short_Long*[TrialPeriod]*[T])
COMPARE ([T]) ADJ(BONFERRONI).
```

The full model shows:

Fixed Effects

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	43.447	58.882	.000
DrugGroup	1	207.407	3.246	.073
Short_Long	1	47.204	2.843	.098
TrialPeriod	4	228.766	1.495	.205
T	1	259.580	4.704	.031
DrugGroup * TrialPeriod	4	219.364	.229	.922
Short_Long * TrialPeriod	4	226.079	1.361	.248
DrugGroup * Short_Long	1	178.020	4.147	.043
DrugGroup * T	1	249.682	4.969	.027
Short_Long * T	1	260.249	2.128	.146
TrialPeriod * T	3	251.725	2.327	.075
DrugGroup * Short_Long * TrialPeriod	4	206.377	2.171	.073
DrugGroup * TrialPeriod * T	2	247.733	.414	.661
Short_Long * TrialPeriod * T	3	248.553	1.099	.350
DrugGroup * Short_Long * T	1	255.120	6.422	.012
DrugGroup * Short_Long * TrialPeriod * T	0	.	.	.

a. Dependent Variable: LogitDiscrim.

There is significant 3-way interaction of Infusion, STM/LTM, and Correct/Incorrect ($F(1, 225.120) = 6.422$, $p < .05$); 2-interaction of Infusion and Correct/Incorrect ($F(1, 249.682) = 2.969$, $p < .05$), Infusion and STM/LTM ($F(1, 178.020) = 4.147$, $p < .05$); main effect of Correct/Incorrect ($F(1, 259.580) = 4.704$, $p < .05$).

To understand the exact differences, we look at post-hoc pairwise comparisons with the Bonferroni correction

Pairwise Comparisons[a]

Short_Long	TrialPeriod	T	(I) DrugGroup	(J) DrugGroup	Mean Difference (I-J)	Std. Error	df	Sig.[e]
STM	1	0	Vehicle	K252a	.308	1.772	245.242	.862
			K252a	Vehicle	-.308	1.772	245.242	.862
		1	Vehicle	K252a	-.341	.760	224.519	.654
			K252a	Vehicle	.341	.760	224.519	.654
	2	0	Vehicle	K252a	-2.153	2.186	247.663	.326
			K252a	Vehicle	2.153	2.186	247.663	.326
		1	Vehicle	K252a	-.436	.751	229.270	.561
			K252a	Vehicle	.436	.751	229.270	.561
	3	0	Vehicle	K252a	1.020	2.392	247.714	.670
			K252a	Vehicle	-1.020	2.392	247.714	.670
		1	Vehicle	K252a	-.007	.720	220.921	.993
			K252a	Vehicle	.007	.720	220.921	.993
	4	0	Vehicle	K252a	.[b]	.	.	.
			K252a	Vehicle	.[c]	.	.	.
		1	Vehicle	K252a	1.193	.738	225.429	.107
			K252a	Vehicle	-1.193	.738	225.429	.107
	5	0	Vehicle	K252a	.[b,c]	.	.	.
			K252a	Vehicle	.[b,c]	.	.	.
		1	Vehicle	K252a	-.127	.684	215.612	.853
			K252a	Vehicle	.127	.684	215.612	.853
LTM	1	0	Vehicle	K252a	7.200*	1.728	262.012	.000
			K252a	Vehicle	-7.200*	1.728	262.012	.000
		1	Vehicle	K252a	-.396	.907	240.647	.663
			K252a	Vehicle	.396	.907	240.647	.663
	2	0	Vehicle	K252a	.[c]	.	.	.
			K252a	Vehicle	.[b]	.	.	.
		1	Vehicle	K252a	1.046	.761	239.487	.170
			K252a	Vehicle	-1.046	.761	239.487	.170
	3	0	Vehicle	K252a	.[c]	.	.	.
			K252a	Vehicle	.[b]	.	.	.
		1	Vehicle	K252a	-.365	.743	234.804	.623
			K252a	Vehicle	.365	.743	234.804	.623
	4	0	Vehicle	K252a	.[b]	.	.	.
			K252a	Vehicle	.[c]	.	.	.
		1	Vehicle	K252a	-1.576*	.790	242.269	.047
			K252a	Vehicle	1.576*	.790	242.269	.047
	5	0	Vehicle	K252a	.[b,c]	.	.	.
			K252a	Vehicle	.[b,c]	.	.	.
		1	Vehicle	K252a	-.916	.746	236.342	.221
			K252a	Vehicle	.916	.746	236.342	.221

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogitDiscrim.

b. The level combination of factors in (J) is not observed.

c. The level combination of factors in (I) is not observed.

e. Adjustment for multiple comparisons: Bonferroni.

We see here that, during STM testing, correct and incorrect trials were not different for the drug and vehicles during any of digging time probe trials, including T1 ($p > .05$). During LTM, however, we found that vehicle had a significantly higher DI than the drug group during T1 for the incorrect T1s only ($p < .05$).

DrugGroup	Short_Long	TrialPeriod	(I) T	(J) T	Mean Difference (I-J)	Std. Error	df	Sig.[e]
Vehicle	STM	1	0	1	-1.370	1.246	246.143	.273
			1	0	1.370	1.246	246.143	.273
		2	0	1	-2.188	1.129	241.304	.054
			1	0	2.188	1.129	241.304	.054
		3	0	1	-.810	2.014	246.938	.688
			1	0	.810	2.014	246.938	.688
		4	0	1	-3.105*	1.117	242.335	.006
			1	0	3.105*	1.117	242.335	.006
		5	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.
	LTM	1	0	1	3.726*	1.539	256.697	.016
			1	0	-3.726*	1.539	256.697	.016
		2	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.
		3	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.
		4	0	1	-1.239	1.532	259.872	.419
			1	0	1.239	1.532	259.872	.419
		5	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.
K252a	STM	1	0	1	-2.020	1.483	247.543	.175
			1	0	2.020	1.483	247.543	.175
		2	0	1	-.471	2.014	246.711	.815
			1	0	.471	2.014	246.711	.815
		3	0	1	-1.836	1.473	246.897	.214
			1	0	1.836	1.473	246.897	.214
		4	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.
		5	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.
	LTM	1	0	1	-3.870*	1.199	251.392	.001
			1	0	3.870*	1.199	251.392	.001
		2	0	1	-1.421	2.064	262.972	.492
			1	0	1.421	2.064	262.972	.492
		3	0	1	.066	1.502	259.595	.965
			1	0	-.066	1.502	259.595	.965
		4	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.
		5	0	1	.[c]	.	.	.
			1	0	.[d]	.	.	.

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Dependent Variable: LogitDiscrim.

c. The level combination of factors in (I) is not observed.

d. The level combination of factors in (J) is not observed.

e. Adjustment for multiple comparisons: Bonferroni.

The post-hoc tests also show that for STM trials, neither the drug or vehicle group had different number of correct and incorrect T1 (*ps* > .05). During LTM

testing, the drug group had significantlyfewer correct trials than incorrect trials ($p < .05$)

Pre-Retrieval infusions Digging Time/Discrimination Index

Data file: TONG_K252aSTMLTMDiggingLongForm-RetrievalOnly.sav
Output file: TONG_Discrim_DiscIndex.spv

```
DATASET ACTIVATE DataSet4.
MIXED DigDiscrim BY Test_Training DrugGroup DigTrial MouseID Trial
 /CRITERIA=CIN(95) MXITER(100) MXSTEP(10) SCORING(1)
SINGULAR(0.000000000001) HCONVERGE(0, ABSOLUTE) LCONVERGE(0,
ABSOLUTE) PCONVERGE(0.000001, ABSOLUTE)
/FIXED=Test_Training DrugGroup DigTrial Test_Training*DrugGroup
Test_Training*DigTrial DrugGroup*DigTrial Test_Training*DrugGroup*DigTrial |
SSTYPE(3)
 /METHOD=REML
 /PRINT=SOLUTION
 /RANDOM=intercept | subject(MouseID)
 /RANDOM=intercept | subject(Trial*MouseID)
 /SAVE=FIXPRED PRED RESID
/EMMEANS=TABLES(DrugGroup*Test_Training*DigTrial) COMPARE (DrugGroup)
ADJ(BONFERRONI)
/EMMEANS=TABLES(DrugGroup*Test_Training*DigTrial) COMPARE
(Test_Training) ADJ(BONFERRONI)
/EMMEANS=TABLES(DrugGroup*Test_Training*DigTrial) COMPARE (DigTrial)
ADJ(BONFERRONI).
```

FULL MODEL:

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	14.000	446.844	.000
Test_Training	1	126.000	103.911	.000
DrugGroup	1	14.000	.047	.831
DigTrial	4	126.000	45.219	.000
Test_Training * DrugGroup	1	126.000	.338	.562
Test_Training * DigTrial	4	126.000	8.867	.000
DrugGroup * DigTrial	4	126.000	.072	.991

Test_Training * DrugGroup * DigTrial	4	126.000	1.441	.224

a. Dependent Variable: DigDiscrim.

Significant two-way interaction of Test_Training and DigTrial, and main effects of DigTrial and Test_Training.

In post-hoc tests:

Test_Training	DigTrial	(I) DrugGroup	(J) DrugGroup	Std. Error	df	Sig.[b]
Training	1	Vehicle	K252a	.111	99.038	.838
		K252a	Vehicle	.111	99.038	.838
	2	Vehicle	K252a	.111	99.038	.133
		K252a	Vehicle	.111	99.038	.133
	3	Vehicle	K252a	.111	99.038	.604
		K252a	Vehicle	.111	99.038	.604
	4	Vehicle	K252a	.111	99.038	.761
		K252a	Vehicle	.111	99.038	.761
	5	Vehicle	K252a	.111	99.038	.900
		K252a	Vehicle	.111	99.038	.900
TestRetr	1	Vehicle	K252a	.111	99.038	.604
		K252a	Vehicle	.111	99.038	.604
	2	Vehicle	K252a	.111	99.038	.327
		K252a	Vehicle	.111	99.038	.327
	3	Vehicle	K252a	.111	99.038	.427
		K252a	Vehicle	.111	99.038	.427
	4	Vehicle	K252a	.111	99.038	.690
		K252a	Vehicle	.111	99.038	.690
	5	Vehicle	K252a	.111	99.038	.528
		K252a	Vehicle	.111	99.038	.528

No significant comparisons

DrugGroup	DigTrial	(I) Test_Training	(J) Test_Training	Std. Error	df	Sig.[c]	95% Confidence Interval for Difference[c]	
							Lower Bound	Upper Bound
Vehicle	1	Training	TestRetr	.099	126.000	.000	-.754	-.363
		TestRetr	Training	.099	126.000	.000	.363	.754
	2	Training	TestRetr	.099	126.000	.000	-.791	-.400
		TestRetr	Training	.099	126.000	.000	.400	.791
	3	Training	TestRetr	.099	126.000	.047	-.393	-.002
		TestRetr	Training	.099	126.000	.047	.002	.393
	4	Training	TestRetr	.099	126.000	.014	-.441	-.050
		TestRetr	Training	.099	126.000	.014	.050	.441
	5	Training	TestRetr	.099	126.000	.390	-.281	.110
		TestRetr	Training	.099	126.000	.390	-.110	.281
K252a	1	Training	TestRetr	.099	126.000	.000	-.835	-.444
		TestRetr	Training	.099	126.000	.000	.444	.835
	2	Training	TestRetr	.099	126.000	.002	-.512	-.121
		TestRetr	Training	.099	126.000	.002	.121	.512
	3	Training	TestRetr	.099	126.000	.606	-.247	.144
		TestRetr	Training	.099	126.000	.606	-.144	.247
	4	Training	TestRetr	.099	126.000	.001	-.520	-.129
		TestRetr	Training	.099	126.000	.001	.129	.520
	5	Training	TestRetr	.099	126.000	.088	-.365	.026
		TestRetr	Training	.099	126.000	.088	-.026	.365

DrugGroup	Test_Training	(I) DigTrial	(J) DigTrial	df	Sig.[c]
Vehicle	Training	1	2	126.000	.080
			3	126.000	.000
			4	126.000	.000
			5	126.000	.000
		2	1	126.000	.080
			3	126.000	.001
			4	126.000	.000
			5	126.000	.000
		3	1	126.000	.000

			2	126.000	.001
			4	126.000	1.000
			5	126.000	1.000
		4	1	126.000	.000
			2	126.000	.000
			3	126.000	1.000
			5	126.000	1.000
		5	1	126.000	.000
			2	126.000	.000
			3	126.000	1.000
			4	126.000	1.000
	TestRetr	1	2	126.000	.027
			3	126.000	.021
			4	126.000	.002
			5	126.000	.012
		2	1	126.000	.027
			3	126.000	1.000
			4	126.000	1.000
			5	126.000	1.000
		3	1	126.000	.021
			2	126.000	1.000
			4	126.000	1.000
			5	126.000	1.000
		4	1	126.000	.002
			2	126.000	1.000
			3	126.000	1.000
			5	126.000	1.000
		5	1	126.000	.012
			2	126.000	1.000
			3	126.000	1.000
			4	126.000	1.000
K252a	Training	1	2	126.000	.000
			3	126.000	.000
			4	126.000	.000
			5	126.000	.000

	2	1	126.000	.000
		3	126.000	.035
		4	126.000	.242
		5	126.000	.005
	3	1	126.000	.000
		2	126.000	.035
		4	126.000	1.000
		5	126.000	1.000
	4	1	126.000	.000
		2	126.000	.242
		3	126.000	1.000
		5	126.000	1.000
	5	1	126.000	.000
		2	126.000	.005
		3	126.000	1.000
		4	126.000	1.000
TestRetr	1	2	126.000	1.000
		3	126.000	.998
		4	126.000	.003
		5	126.000	.008
	2	1	126.000	1.000
		3	126.000	1.000
		4	126.000	.200
		5	126.000	.389
	3	1	126.000	.998
		2	126.000	1.000
		4	126.000	.408
		5	126.000	.748
	4	1	126.000	.003
		2	126.000	.200
		3	126.000	.408
		5	126.000	1.000
	5	1	126.000	.008
		2	126.000	.389
		3	126.000	.748
		4	126.000	1.000