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**Olfactory Conditioning in Cockroaches**

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This experiment is designed to test whether cockroaches can be conditioned using olfactory cues. We used vanilla and lavender essential oils, but this could be modified to test any scents that you have available. You will first assess the scent preferences of the roaches in a preference test, then reward them for choosing the scent that is not preferred and finally see if they retain their acquired scent preference when no rewards are present. This experiment is more extensive than our others, so we consider this an advanced experiment. However, you could shorten the memory delay intervals to scale down the difficulty of this task.

Replication of:

Balderrama, N. (1980). One trial learning in the American cockroach, *Periplaneta americana*. Journal of Insect Physiology, 26(8), 499-504.

Sakura, M., & Mizunami, M. (2001). Olfactory learning and memory in the cockroach *Periplaneta americana*. Zoological Science, 18(1), 21-28. doi:10.2108/zsj.18.21

**Method**

**Materials**

Sugar solution 50% (w-w) will be used as the positive reinforcer during training, prepared with D(+) sucrose. Saline solution 20% (w-v) will be used as the aversive stimulus, prepared with sodium chloride, aka salt. Vanilla essential oil (*Vanilla planifolia*, Gya Labs) and lavender essential oil (*Lavendula angustifolia*, Apothecary) will be used as olfactory discriminative stimuli. The brand of the essential oils likely does not matter. However, we used lavender rather than the peppermint that was originally used in the papers we are replicating. We found that our roaches would not even go near peppermint. We suspect the scent was too strong.

Training periods will be conducted in an experimental chamber. We use a 11.75 x 7.75 x 8 in plastic tank. These are available at most pet stores and are relatively inexpensive. No substrate is provided during testing or training periods. At one end of the experimental chamber is the training apparatus, consisting of a [3D-printed plastic ‘platter’ with two circular depressions](https://roach-lab.squarespace.com/s/choice-platter.stl). The depressions are sized to fit a [Publix water bottle cap](https://www.publix.com/pd/publix-water-spring/RIO-PCI-103276?ch=9.2.8.), but other small bottle caps may fit. The odor source is a piece of filter paper inserted into the cap, saturated with the essential oil. The bottle cap is topped with a [3D-printed plastic cap featuring four holes](https://roach-lab.squarespace.com/s/bottle-cap-top.stl), allowing the subject to smell but not otherwise access the essential oil. The center of the cap also features a liquid reservoir that can hold up to 1 mL of the sugar or saline solution. Lastly, one of two paper ‘posters’ are be placed on the wall of the experimental chamber during training. These posters have high-contrast black-and-white patterns, either stripes or dots, and can serve as conditional discriminatory stimuli in later experiments.

Additionally, we place a piece of paper with subdividing lines under experimental chamber. This enables experimenters to more accurately code where the roach spends time either live or from video. If you are going to use video coding, we highly recommend checking out [BehaviorCloud](https://www.behaviorcloud.com/academia.html). This software, that is free for limited use, can automatically track where the roaches are in the experimental chamber.

**Experimental Procedures**

**Preference Assessment.** A free-operant preference assessment will be conducted prior to training.The purpose of the preference assessment is to determine if the subjects have an innate or pre-existing preference for either of the two target odors before training. Before every preference assessment, the interior of the experimental chamber should be cleaned to remove any residual scents or excreta from previous sessions. Two pieces of filter paper will be soaked in the extracts, one lavender, one vanilla, and placed in the odor sources. The caps will then be fitted onto the odor source. No liquid solution of any kind will be placed in the reservoirs. The odor sources will then be placed on the platter, and the platter will be placed at one end of the experimental chamber. No posters will be placed in the experimental chamber during the preference assessment. A cockroach is then placed in the opposite end of the experimental chamber and allowed to freely move within the chamber for 3 min. After the initial 3 min has elapsed, the platter with the odor sources will be rotated in order to change the relative positions of the vanilla and lavender essential oils. The cockroach is then allowed to freely move within the chamber for another 3 min. For both preference assessment periods, two dependent variables will be measured: the latency to approaching each odor source, and the cumulative duration of time spent visiting each odor source. Visiting is operationally defined as when the cockroach’s head is directly above the cap of the odor source or inserted in the hole of the cap. After the time has elapsed, the roach is briefly removed from the experimental chamber. The experimenter will need to determine which odor source the roach spent more time with. That odor will be the roaches preferred odor. The other odor is the non-preferred odor.

**Training.** The training session will begin as soon as possible after the preference assessment is completed. Before returning the cockroach to the experimental chamber: (a) the odor source platter will be rotated, (b) the reservoirs of the odor sources will be filled with sugar or saline solution, and (c) a poster will be placed in the enclosure. The specific poster used, stripes or dots, will be counterbalanced across individuals. The sugar solution is placed in the reservoir of the less preferred essential oil, and the saline solution will be placed with the preferred. In the event of equal/no preference, the sugar solution will be placed with the lavender essential oil, as the roaches generally seem to prefer vanilla. The cockroach is then placed in the experimental chamber on the side opposite of the platter. Upon approaching an odor source, the cockroach will be able to drink the solution from the reservoir; cockroaches will be limited to a total of 20 s access to the sugar solution, after which the cockroach will be removed from the experimental chamber, the reservoirs will be refilled, and the platter will be rotated before the cockroach will be re-introduced to the experimental chamber. Training trials will last until either the cockroach completes 20 s of drinking the sugar solution, or 4 min have elapsed, whichever occurs first. One trial block will be administered per cockroach per day. A trial block will consist of two trials, meaning training sessions will last a maximum of 8 min. Cockroaches will be divided into three groups: (a) those that receive one trial block of training, (b) two trial blocks, or (c) seven trial blocks.

**Retention Testing.** After completing the preference assessment and training, the cockroaches will undergo three retention tests. The retention test will have identical procedures to the free-operant preference test. The interval between the last training session and the retention test will be referred to as the retention interval (RI). Retention tests will be conducted one day, two days, and seven days after the cockroach completes its training trial block(s). However, feel free to modify your retention intervals as needed.

**Re-Training.** After completing the three retention tests, the cockroaches will undergo a second training regimen. The procedures will be identical to the first training program, with two exceptions: (a) the ‘opposite’ poster will be placed in the enclosure (e.g., if the striped poster was presented in the initial training sessions, the dotted poster will be used in the re-training sessions), and (b) the placement of the solutions will be reversed, with the sugar solution being placed in the reservoir of the vanilla extract odor source, and the saline solution being placed in the reservoir of the lavender extract odor source. Cockroaches will remain in their assigned groups from the first training program (i.e., 1-block, 2-block, or 7-blocks of training).

**Discrimination Testing**. After completing the second training program, the cockroaches will undergo a pair of discrimination tests. The procedures will be identical to those in the retention assessment, with two exceptions. First, the two discrimination tests will be conducted back-to-back, with an interval of one day from the completion of the re-training. Second, the posters will be present during the discrimination tests, with the stripes poster being present for one test, and the dotted poster being present for the other. The order of presentation will be counter-balanced across cockroaches.