



Jul 23, 2020

Irritability-like behavior testing with the bottle brush

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ABSTRACT

Irritability Protocol

We use the bottle-brush test to test for irritability-like behavior see previous studies for examples (Kononoff et al., 2018, Kimbrough et al., 2017). The bottle-brush test uses a bottle brush to measure aggressive and defensive responses from the rats.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kimbrough, A., de Guglielmo, G., Kononoff, J., Kallupi, M., Zorrilla, E.P., George, O. (2017) CRF1-dependent increases in irritability-like behavior during abstinence from chronic intermittent ethanol vapor exposure. *Alcoholism: Clinical and Experimental Research*, 41(11), 1886-1895. PMID:28833238

PROTOCOL CITATION

Adam Kimbrough, Olivier George 2020. Irritability-like behavior testing with the bottle brush. **protocols.io** <https://protocols.io/view/irritability-like-behavior-testing-with-the-bottle-8wxhxfn>

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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CREATED

Oct 31, 2019

LAST MODIFIED

Jul 23, 2020

PROTOCOL INTEGER ID

29367

GUIDELINES

Specific Behaviors Examined:

Aggressive responses:

smelling the brush: the rat moves close to the brush and smells the brush

immediate biting the brush: during the initial movement of the brush toward the rat and the rotating against their whiskers the rat bites the brush

delayed biting of the brush: during the return of the brush toward the back of the cage or while the brush is hanging vertical before removal the rat bites the brush

boxing the brush: the rat uses their paws like 'boxing gloves' to bat the brush away from them while it is being rotated against their face

following the brush: the rat follows the brush to where the brush is hanging at the other end of the cage before removal

exploring the brush: the rat manipulates the brush by grabbing, pushing, pulling, or climbing the brush

Defensive responses:

escaping from brush: the rat moves their body away from the brush while it approaches them or is rotated against them in order to avoid/minimize contact

digging: the rat digs the bedding. This can occur during or between trials.

jumping: the rat jumps up or sideways in a startled manner

climbing: the rat puts their paws up in an extended position towards the upper walls of the cage during the period while the brush is approaching them or being brushed against them

defecation: the rat defecates during/between a trial

vocalization: the rat vocalizes as the brush approaches or is brushed against them

grooming: the rat grooms themselves. This can occur during or between trials.

MATERIALS TEXT

Materials:

People- 2-3 Observers and 1 Brusher

Rats

Clean rat cages

Bottle brush

Specially designed filter top lid (with filter removed and portion of plastic grid removed for movement of brush)

Scoring sheets

BEFORE STARTING

Note, when applicable observers should be blind to treatment conditions of the animals

Note, it is recommended that people work with others who have scored/brushed before to learn the procedure and what visually each behavior entails

1 Procedure:

Testing usually begins during the dark cycle of the animals under dark conditions with a red light for researchers but may vary depending on the experiment.

A session consists of 10 trials in a clean plastic cage (26.67 cm × 48.26 cm × 20.32 cm; Ancare, Bellmore, NY, USA) with fresh bedding.

A trial begins with the rat positioned at the back of the cage and the rat should be moved to the back of the cage if not there, trials occurring with the rat starting elsewhere should be disregarded.

The person performing the brushing inserts the bottle-brush into the cage from the front, rotating towards the rats' whiskers for approximately 3 seconds. The brush should make contact with the rat for approximately a second on the whiskers.

The rotating brush is then retreated to the front of the cage over the course of a second.

The brush then is hung vertically for approximately 2 seconds (without rotating) before being removed entirely from the cage. This step and the previous step allow for the rat to follow the brush to the resting point and bite, paw at and inspect the brush if they choose to.

Removal of the brush from the cage indicates the end of a trial, which should be repeated 10 times. After a 5 second gap the trial is repeated.

During the trial the 2-3 observers identify behavioral responses that are categorized into aggressive and defensive. Each behavior that occurs in a given trial is recorded between the trials on scoresheets. A behavior occurring in an individual trial results in a tally mark for that behavior in that given trial on the scoresheet.

At the end of the sessions the total score for each behavior (and aggressive or defensive behaviors) are tallied for each rat and the average across observers is taken.