



STANDARD OPERATING PROCEDURE

TITLE: Y-maze Spontaneous Alternation Test

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the Y-maze Spontaneous Alternation Test. Spontaneous Alternation Tests are used to evaluate exploratory behavior in mice and rats. Brain areas involved in this test include hippocampus, septum, basal forebrain, and prefrontal cortex. As this is a general description of standard materials, test settings, and procedures, variations may be made to fit specific needs.

Materials

- *Subjects:* any strain of rats or mice. No prior training is required, though subjects should be acclimated to testing environment and experimenter before testing.
- *Apparatus:* Y-shaped maze with three opaque arms spaced 120° apart.
 - *Large Y-maze:* three 40 x 8 x 15cm arms.
 - *Small Y-maze:* two 15.24 x 7.62 x 12.7cm arms, one 20.32 x 7.62 x 12.7cm arm.
- *Overhead camera:* mounted to ceiling directly above apparatus.
- *Software:* automated tracking system (ex. EthoVision).
- *Privacy blinds:* placed around apparatus to eliminate external room cues.
- *Standing lamps with white light bulbs (4):* placed at corners outside privacy blinds pointed away from apparatus.
- *Light meter:* used to measure illumination in arms of apparatus.
- *Virkon:* used between trials to eliminate visual and olfactory residue in arena.

Test Settings

- *Lighting:* comparable illumination in all arms of apparatus.

Detailed Standard Operating Procedure

Before testing:

- *Acclimation:* subjects in home cage are placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing.

TITLE: Y-maze	<i>Stanford Behavioral and Functional Neuroscience Laboratory</i>	
CATEGORY: Behavioral Assay	Page 2 of 3	Version 4.0

- *Subject training*: none required.

Testing procedures:

- Animal is placed just inside arm B facing away from center and allowed to move through apparatus for 5 or 8min (small or large Y-maze, respectively) while being monitored by automated tracking system. Trial begins immediately and ends when defined duration has elapsed.
- Scoring consists of recording each arm entry (defined as all four paws entering arm). Scoring should be conducted live if possible by monitoring movement through apparatus via computer screen.
- Animal is returned to home cage and number of fecal pellets is recorded.
- Arena is cleaned with Virkon between trials.

Data Analysis

- Total number of entries should not include first recorded arm (always B).
- **Number of triads = (Total entries – 2)**
Triad (set of three letters) containing all three letters is scored as alternation.
- **Percent alternation = [(# of Alternations/ Total # of triads) *100]**

TITLE: Y-maze	<i>Stanford Behavioral and Functional Neuroscience Laboratory</i>	
CATEGORY: Behavioral Assay	Page 3 of 3	Version 4.0

Example Y-maze Score Sheet

Study #:	Y-maze Group:	Date:
Test #:		Initials:

Animal ID# _____	Total Entries =	# of Triads =	Pellets =

Animal ID# _____	Total Entries =	# of Triads =	Pellets =

Animal ID# _____	Total Entries =	# of Triads =	Pellets =

Animal ID# _____	Total Entries =	# of Triads =	Pellets =