



STANDARD OPERATING PROCEDURE

TITLE: T-maze Spontaneous Alternation

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the T-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:* T-shaped maze with three 30 x 10 x 20cm arms.
- *Overhead camera:* mounted to ceiling directly above apparatus.
- *Software:* automated tracking system (ex. EthoVision).
- *Privacy blinds:* placed around apparatus at least 1ft from maze to eliminate external room cues.
- *Visual cues (2):* paper printouts of shapes attached to blinds and clearly visible from arms.
- *Standing lamps with white light bulbs (2):* placed outside privacy blinds pointed away from apparatus.
- *Light meter:* used to measure illumination in arms of apparatus.
- *Rolled paper towels:* used to stimulate sluggish animals.
- *Virkon:* used between trials to eliminate visual and olfactory residue in arena.

Test Settings

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

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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.
- *Subject training*: none required.

Testing procedures:

- Left and right arm gates are opened, animal is placed in start arm, and recording begins.
- Start gate is opened quietly when animal is facing away from goal arms and timing begins.
- When all four paws enter one arm, other arm gate is closed and arm entry is recorded as R or L.
- Arm gate is closed upon return from arm and start gate is closed upon reentry into starting arm. If animal is motionless in goal arm for longer than 90s, rolled paper towel is used to gently touch mouse and guide towards start arm.
- Procedure is repeated for total of 11 trials. Recording and timing end once animal returns to start arm in trial 11. If animal does not complete 7 trials within 15min, trial should be stopped and animal should be retested after 1hr. If on second trial animal again does not complete 7 trials within 15min, trial should be stopped and animal should not be tested third time.
- Animal is returned to home cage and number of fecal pellets is recorded.
- Arena is cleaned with Virkon between trials.

Data Analysis

- The following parameters are collected for analysis:
 - Percent alternation (see below)
 - On each day
 - On average of all days
 - Percent of right/left turns
 - Number of pellets
 - Total duration
- **Percent alternation = [(Score/10)*100]**
 Score is determined by counting number of alternations made, with maximum of 10 alternations for 11 trials.

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Example T-maze Score Sheet

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

Animal ID#
Cage#

Trial	Arm
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	

Animal ID#
Cage#

Trial	Arm
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	

Animal ID#
Cage#

Trial	Arm
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	

Animal ID#
Cage#

Trial	Arm
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	

Animal ID#
Cage#

Trial	Arm
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	

Score	
Time	
Pellets	
Left counts	

Score	
Time	
Pellets	
Left counts	

Score	
Time	
Pellets	
Left counts	

Score	
Time	
Pellets	
Left counts	

Score	
Time	
Pellets	
Left counts	