



# STANDARD OPERATING PROCEDURE

**TITLE: Novel Object Recognition**

**CATEGORY: Behavioral Assay**

## Introduction

**Goal:** This document aims to provide the reader information on how to conduct the Novel Object Recognition Test. The test is used to evaluate hippocampal function and recognition memory, specifically working memory and spatial navigation. 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 mice. Subjects require training and should be acclimated to testing environment and experimenter before testing.
- *Apparatus:* 40 x 40cm open field chamber divided into two 20 x 40cm arenas.
- *Visual cues (4):* placed on north and west walls of left arena as well as south and east walls of right arena.
- *Objects (8):* four each of Objects A and B, which are similar in height and volume but different in shape and texture.
- *Overhead camera:* mounted to ceiling directly above apparatus.
- *Software:* automated tracking system (ex. EthoVision).
- *Privacy blinds (2):* placed completely surrounding apparatus approximately 1ft from perimeter to eliminate external room cues.
- *Standing lamps with white light bulbs (4):* aimed away from apparatus to provide diffuse light.
- *Light meter:* used to measure illumination in apparatus.
- *20% ethanol:* used between trials to eliminate visual and olfactory residue in arena.

## **Test Settings**

- *Lighting:* dim, consistent illumination throughout apparatus.

<b>TITLE: Novel Object Recognition</b>	<i>Stanford Behavioral and Functional Neuroscience Laboratory</i>	
<b>CATEGORY: Behavioral Assay</b>	Page 2 of 5	Version 4.0

### 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*:
  - 1. Training Part 1**
    - Two identical objects of type A are taped to floor of each arena 5cm from east or west wall and 10cm from south wall (left arena) or north wall (right arena) (see Figure 1).
    - Animal is placed in center of arena and allowed to explore for 10min (NOTE: animal should be placed in same arena, left or right, that it was habituated to). With two arenas, two animals may be run simultaneously. Trial begins immediately and ends after defined 10min duration has elapsed.
    - Arena and objects are cleaned with 20% ethanol between trials.
  - 2. Training Part 2**
    - Each animal should have 3hrs between start of Training Part 1 and start of Training Part 2.
    - Object A closest to outer wall of arena is moved so that it is 10cm from north wall (left arena) or south wall (right arena) (see Figure 2).
    - Animal is placed in center of arena and trial is run as in Training Part 1.

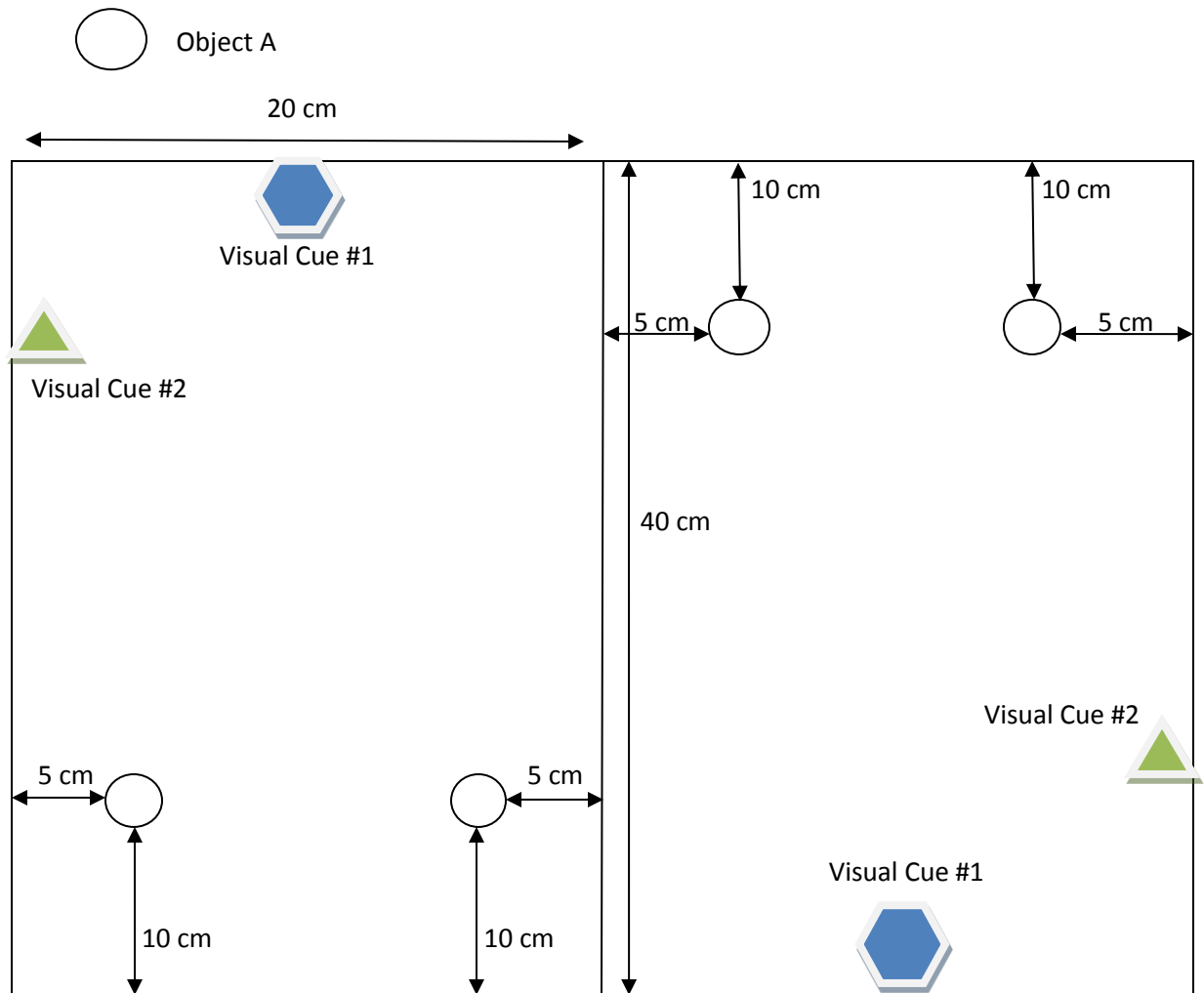


Figure 1. Training Part 1 Setup

<b>TITLE: Novel Object Recognition</b>	<i>Stanford Behavioral and Functional Neuroscience Laboratory</i>	
<b>CATEGORY: Behavioral Assay</b>	Page 4 of 5	Version 4.0

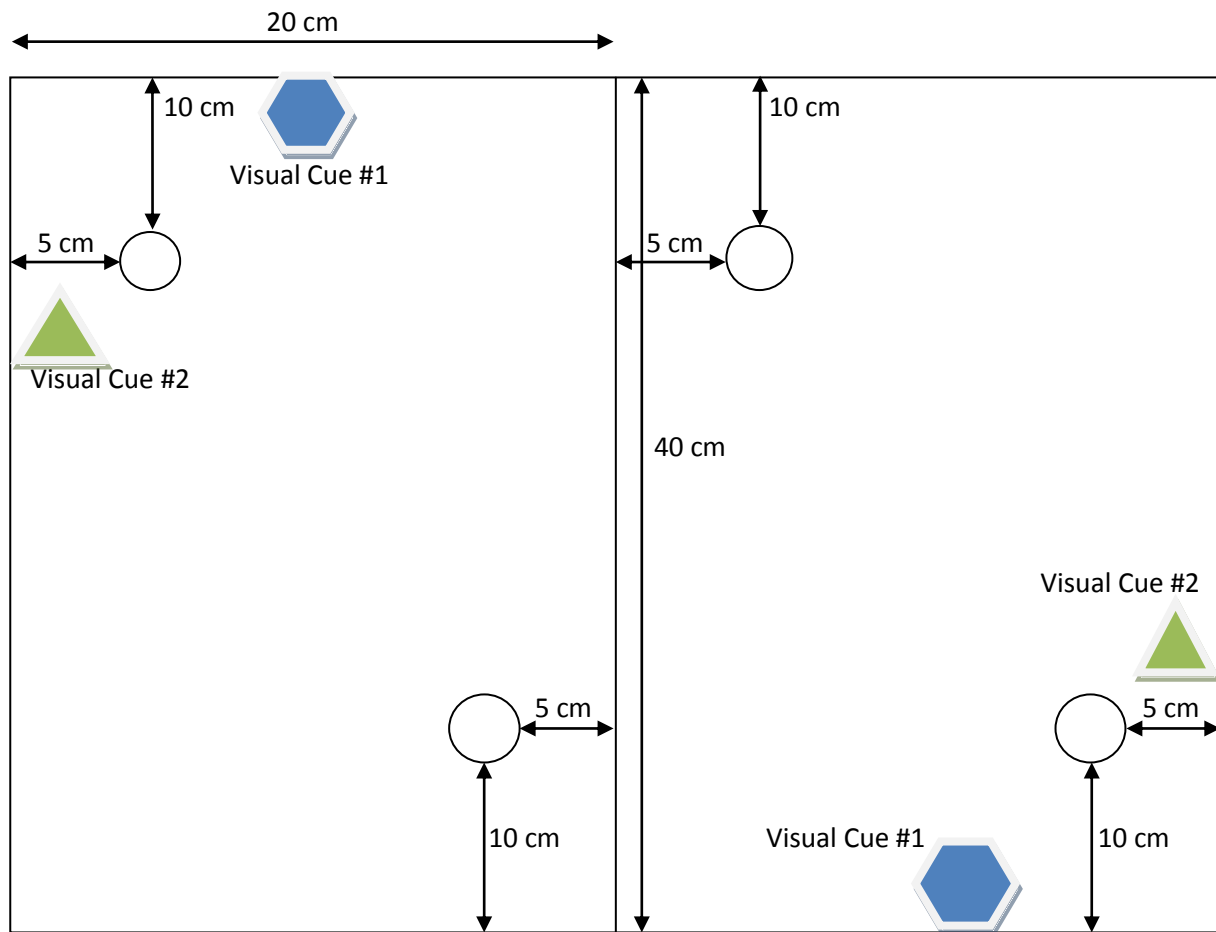


Figure 2. Training Part 2 Setup

Testing procedures:

- Each animal should have 24hrs between start of Training Part 2 and start of testing.
- Object A that was not moved in Training Part 2 is replaced with novel object (Object B) (see Figure 3).
- Animal is placed in center of arena and trial is run as in Training Parts 1 and 2.

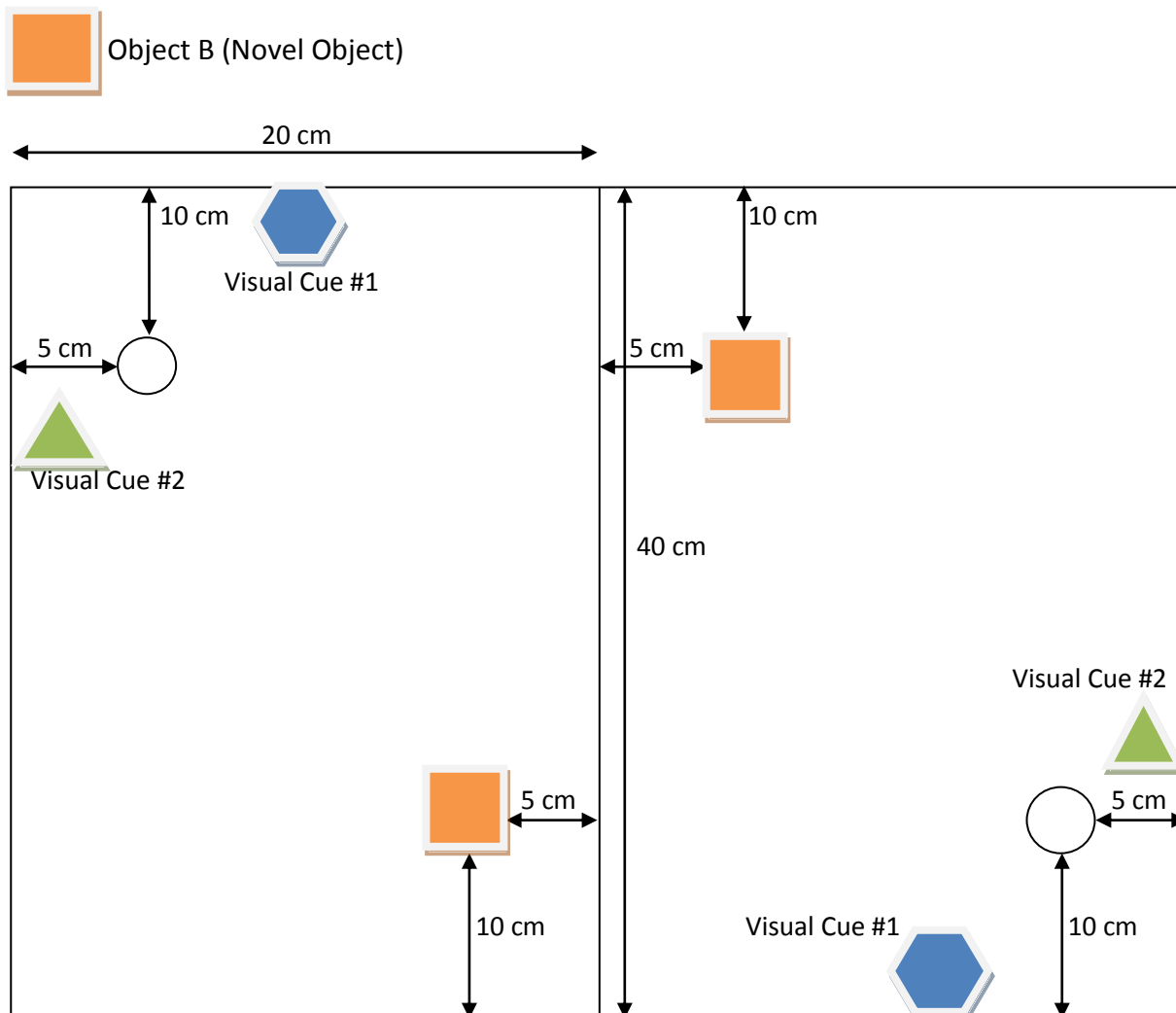


Figure 3. Novel Object Recognition Testing Setup

**Data Analysis**

- Interaction with each object (defined as sniffing and/or head within 1cm of object) is collected for analysis.