



**AfaSci Research Laboratories**  
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### **SmartCage testing protocols**

AfaSci, Inc.

All modular SmartCages can do the first 7 tests listed below.

For tests 8 – 14, a special modular SmartCage is required. This applies to the Rotarod module for the Rotarod test, the Wake-up for automated sleep deprivation, Food Controller for Feeding behavior, Stimulation module for Drinking behavior, Vogel conflict drinking test, Foot drop/fault/drop, Step-through passive avoidance and Tone- and Contextual-conditioning fear tests.

In general, the shock protocols are arranged to be performed at the late stage as shocks generate longer-term effects on consequent behavior tests.

1. Locomotion, active/inactive and rearing (1 - 5 days). The first day is acclimation. Different mice/strain/genotypes can have different ability to accommodate to new an environment which could be biological meaningful itself. The second and third days are active/inactive cycles. If drug treatment is applied, there could be baseline, drug treatment, and recovery each day.
2. Sleep/Wake cycle (1 - 5 days) is monitored using the Piezo-pad while locomotion is simultaneously recorded. **The sleep is indirect and total sleep measurement.**
3. Tail suspension (6 min) to assess depression-like behavior using the piezo-pad.
4. Light/dark for anxiety testing (10 min) using a fresh homepage for measure anxiety-like behavior.
5. Conditional place preference (10 min) test using a central divider with different color or textures between two compartments.
6. Social interaction test (30 min)
7. Social recognition memory test (30 min)
8. Rotarod: This test requires daily Rotarod training 3 times each day and usually training over 3 - 4 days reaches a performance plateau and the drug test.
9. Wakeup module for automated sleep deprivation (4 – 6 h sleep deprivation and consequent 1 – 2 days recording for monitor rebound sleep).
10. Feeding behavior (1 – 5 day) can incorporated into the Locomotion experiment (Protocol 2).
11. Drinking behavior (1 – 5 day) can incorporated into the Locomotion experiment (Protocol 1).
12. Vogel conflict drinking test (10 – 40 min). Used to create anxiety model and test anxiolytic effects of drugs.



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13. Foot fault/drop (5 min). Used to assess sensory and coordination ability in walking using the metal mesh pad.
14. Step-through passive avoidance test (5 min). Training session 5 min. Retention time depends on the experiment design, usually in 24 hrs which allow to conduct the memory test in the same circadian time as the training session.
15. Tone-conditioning fear test (5 min) using the metal grid pad. Pre training, a baseline session (5 min) can be recorded and then followed by the foot shocks are paired with tone (5 shocks in 5 min). The tone-conditioning fear memory can be tested 24 hrs after the foot shocks. The animal is placed into a fresh homecage. The first measure assesses free movement for 5 min as new baseline, following by exposure to tones (5 tones in 5 min). The traveling distance, speed and rearing in two baselines and with tones (one day or longer aftershocks) are measured using the SmartCage. Please try to test at the similar circadian time. Tone-conditioning fear test indicates hippocampus-independent memory.
16. Contextual-conditioning fear test (5 min) using the same metal grid pad. In the training session, the foot shocks are paired with tone. The fear-conditioning memory can be tested after the tone-conditioning fear test. Contextual fear by placing the animal back to the same foot shock setting but without shocks or tones. The traveling distance and speed in baseline (the same setting prior to shocks) and one day or longer (1 – 2 weeks) after shocks. Please try to test at the similar circadian time. Contextual-conditioning fear test reflects hippocampus-dependent memory.