

# Timed Behaviors in Mice

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## ABSTRACT

The mouse has an extensive capacity to modulate timed responses and arm movements according to different environmental conditions and different genetic background may affect this ability. Recently, we have characterized a number of quantitative and qualitative meaningful endophenotypes related to mouse timing mechanisms and different motor actions. We have further investigated timing and motor behaviors in mice by acute experiments and long-term monitoring. Novel technological advances allowed us to simultaneously monitor brain and muscle electrical activity, spontaneous and conditioned behaviors as well as metabolic profiles during 24-hours in testing and home-cage environments. We have used these new technologies to screen for novel mutants and to characterize existing mouse lines.

## Author Keywords

Timing, reaching and grasping, motor behaviors.

## INTRODUCTION

A number of functions in organisms require accurate temporal processing of environmental inputs in order to orchestrate the appropriate output. Organisms can process temporal information and generate responses across a wide range of timescales (microseconds to years). The circadian system is not the only biological clock in the organism. Timing is central to many cognitive functions and motor actions. Many neuropsychiatric and neurological disorders present with timing deficits that lead to cognitive or motor symptoms. This shows the fundamental role of our ability to localize ourselves in time. Studies on conditioned behaviors in humans and animals have demonstrated that subjects retain a cognitive biological clock, which allows them to store memories of the duration of an inter-event

interval, and subsequently to recall this information to determine the time of the conditioned response. In this respect, a crucial integrative property of the acting brain is to execute goal-directed motor actions with a proper timing and with a meaningful value.

## METHODS

Recently, we have developed two main automated instruments that allow us to study timing and motor control in mice under the manipulation of biological (e.g. gene) and behavioral (e.g. risk assessment) variables.

### *Timing Screen*

In this task, conditioning is directly evident in the timing of the conditioned response (peak procedure test). In particular, we have trained mice to collect a reward (a food pellet) at a specific time interval following a light event (20 seconds) in the home cage. We then use the timing of the mouse's response during a probe trial, in which no food reward is given, as a measure of learning. By using an automated system, we were able to test the mice at night, their normal period of activity, thus not effecting their natural sleep and/or daily rhythms.

### *Electrophysiological Correlates of the Timing Switch Task in Home-Cage Environment*

This test has been developed by Balci et al. (Balci et al., 2008) in mice. Here mice are trained to perform in a timing task. Respect to other timing tests (e.g. the peak procedure test), the switch task requires for the mouse to judge when it is the optimal time to switch from one feeder to another within the cage. In particular, mice are asked to anticipate the appearance of a reward at 1 of 2 locations at each trial. On a fraction of the trials, the reward pellet comes in location "A" after a short latency (e.g. 3 seconds). On the other trials, the pellet is obtained after a long latency (e.g. 9 seconds) at the location "B". It has been shown that mice learn very fast that it is convenient to wait at the short-latency location. If and when they reckon that the short latency has passed, they decide to leave the location "A" and to move (switching) to the location "B" (Balci et al., 2008). If they switch too soon or too late for those long-latency trials, the mice end up with no payoff. Interestingly, by changing the probability of short trials, the optimal

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switch time changes. The test allows us to test the timing learning of a mouse but also to quantitatively assess how this is performed under risk. The optimal performance at this test implies that the animal has an accurate representation of both endogenous and exogenous uncertainty of the temporal environment. We are interested to test the electrophysiological correlates under temporal uncertainty in mice. Furthermore, we test the relation between timing learning and sleep homeostasis. In doing that, we monitor EEG, muscle and temperature over long periods of time. For this study we use a wireless monitoring system (DSI) consisting of implantable transmitters that measure in mice physiological parameters such as: EEG, EMG, activity and body temperature. The telemetry system allows mice to move freely with no constrictions due to a cable. In fact this strategy allows us to subject mice to automated training in the home cage without any interference from the cable.

#### *Home-Cage Automated Phenotyping*

Because the existing protocols in timing learning are all very time-consuming and with a considerable variability due to the repeated daily sessions for the mouse in a different environment compared to its home-cage, we decided to invest efforts in refining the procedure. To respond to this necessity, we have developed a novel behavioural device which allows testing mice within their home-cage.

#### *Reaching and Grasping Motor Behaviors*

Reaching and grasping are fundamental goal-directed movements across many species, included mice. However, the use of rodents to investigate the mechanisms of reaching and grasping has been limited in genetics. Recently, we have characterized a number of quantitative and qualitative fine motor phenotypes that constitute significant properties of the mouse motor control system. In addition, in our lab we are exploring the possibility of testing specific aspects of arm movement and to study their significance when testing cognitive functions in mice.

#### **Subjects**

We decided to subject a number of mutant lines (circadian and cognitive mutants) and wild-type mice to cognitive timing tests and reaching and grasping tests to explore the possibility that gene mutations affects the timing learning ability of mice at a different timescale.

#### **RESULTS**

Within our studies we characterised a number of timing phenotypes in novel mouse models (e.g. within ENU mutagenesis screens) and in existing mouse mutants. In particular, we created a novel mouse model (*Nosy*) with a cognitive timing delay. We have identified phenodeviants for timing phenotypes in the G3 progeny of ENU mutagenised mice. These mice show an abnormal delay for response in the probe trials (in which no food reward is given), such that the mutant has a 50 second delay,

compared to the 20 second delay seen in wildtype mice (3 out of 32 mice had the phenotype). Initial inheritance testing of this pedigree, indicates a recessive mode of inheritance with incomplete penetrance.

For what regards the motor phenotype issue, we have developed a test, the MoRaG test, that has proven to be successful and rapid for detecting abnormalities/differences in inbred strains, genetically-modified and aged mice (Neuroscience. 2007 Jul 13;147(3):573-82.). Moreover, the test has well served to the characterisation of novel mouse models for hereditary and motor neuropathy (Dis Model Mech. 2009 Jul-Aug;2(7-8):359-73.) and for pathology affecting the strength in limb movement (PLoS One. 2010 Feb 9;5(2):e9137).

#### **CONCLUSION**

To our knowledge, the mutation carried by the *Nosy* line, would be the first genetic locus found to underlie timing learning in mice. Whether such an internal timing mechanism resembles a clock-like oscillator (Church, 1984) or is distributed over a cortical network (Karmarkar and Buonomano, 2007) is still debatable. A few studies have investigated the relationship between circadian clock mechanisms and timing learning, concluding that the two clock systems are independent (Cordes and Gallistel, 2008). However, the question has not well been addressed yet at the molecular level.

By using our Automated Nose Poking prototype we have observed a remarkable reduction of the time of training for each animal. In particular by using our set-up, mice reach a *steady state performance* within 10-15 days of training compared to a 1-month standard protocol.

The detection of nose poking behaviour in conditioned behaviour paradigms has become a common procedure to study cognitive processes in mice. Our study represents an optimisation of this procedure in mice.

Regarding the reaching and grasping experiments, we have observed that attention plays a role in the performance of arm movements in mice improving/diminishing (according to specific compounds) the accuracy to reaching behaviours. An ongoing *in vivo* electrophysiological investigation is now focusing on timed combination of muscle activation as a requirement for developing an appropriate motor learning in mice.

#### **Ethical Statement**

An authorized ethical committee (by the Italian law) has approved the experiments.

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