A new automated system to study discriminative learning for auditory cues

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The ability to discriminate spectral and temporal characteristics of auditory stimuli is an important factor for animals in coping with the environment. However, studying of these processes in laboratory rodents is associated with technical problems. Until now the main behavioral paradigm to study auditory discrimination in laboratory rodents was differential fear conditioning which is strongly aversive and could not be performed in a home cage. To deal with this challenge we have designed a new behavioral paradigm to study ability of mice to discriminate between auditory stimuli using the automated Novel Tune Cage system (New-Behavior; http://www.newbehavior.com). In this paradigm, a group of mice lives in a regular cage connected by a tunnel to a sound-proof chamber (see Figure 1A). Inside the chamber, there is an antenna reading the individual transponder code assigned to each mouse. The shape of the access tunnel restricts the entrance to the chamber for a single mouse only. The water bottles are available only for mice in the chamber, and the animals have to perform a nose-poke in order to open small motorized doors; the nose-pokes are registered by photo sensors.

After a few days of adaptation to the regular cage, the learning procedure with auditory stimuli was started. Tone bursts with 8 kHz frequency ("standard sound") were presented when the mouse was recognized by the sensor during 4 days. Next 4 days two tones were presented randomly. The 75 % of visits were accompanied by the same standard tone and 25 % with another one, "deviant". Deviant was presented either as continuous tone of 11.4 kHz (first experiment, n = 11 mice) or as tone bursts with 11.4 kHz (second experiment, n = 9 mice). For the both experiments each nose-poke performed during the presentation of the deviant tone was punished by an air-puff. At the time of standard sound any nose-poke was opening door and providing access to water. So, depending on the sound presented different module either opening the doors and allowing to drink or activating the punishing air-puff was turned on (see Figure 1B, C).

No changes in general activity of mice were observed after introduction of the standard sound in both experiments. The first "deviant" visit of each mouse was always followed by a nose-poke reaction and hence it was always punished by an air-puff. But when standard and deviant sounds differed in frequency as well as in temporal structure (first experiment) each mouse had only a few deviant visits finished with a nose-poke at the end of the first day of the discriminative procedure. Also, average duration of the deviant visits was significantly lower comparing to the duration of "standard" visits. These results have shown that an unknown sound when paired with aversive stimulus, such as the air-puff, becomes significant for the mice. The animals start to recognize the novel sound as unsafe, differentiate it from the familiar sound and, as a result, develop appropriate behavioral strategy to avoid the punishment. Moreover, it appeared that the temporal structure of auditory signal may be very important for mice, as in second experiment (when two sounds had the same temporal structure and were different only in frequency) only 5 from 9 mice in a group were able to discriminate between the auditory cues. The rest being unable to escape punishment stopped to perform nose-poke reaction in time of deviant as well in time of standard sound soon after discriminative learning procedure was started. So, analysis of general activity shown phenomena of generalization for those mice who had no success in discriminative task.

We conclude that Novel Tune Cage is an effective behavioral system to study auditory discriminative learning in mice. The system allows also revealing the individual differences in learning capacity and may be useful to study the impact of particular genes on recognition of auditory cues.

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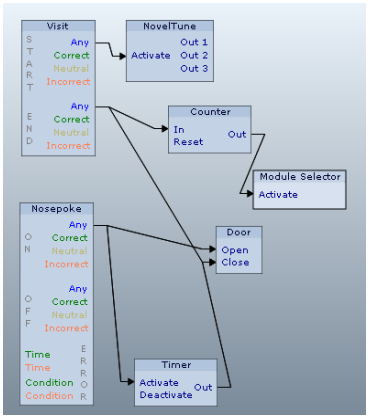
A

B



C

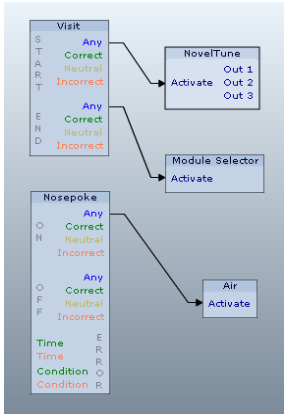


Figure 1. *The general view of Novel Tune Cage (A); modules for "standard" (B) and "deviant" (C) visits during the discrimination learning procedure.*