Fully Automated 24/7 Behavioral Screening for Mutations in Targeted Cognitive Mechanisms in the Mouse

C.R. Gallistel, A.P. King, A.M. Daniel, E.B. Papachristos, and F. Balci
Department of Psychology & Center for Cognitive Science, Rutgers University, New Brunswick, NJ, USA,
galliste@ruccs.rutgers.edu

Current screens for memory defects in mutant mice, such as the Morris water maze, generally proceed on the assumption that memory impairment will be manifest in an altered rate of learning. The rate of learning in mutant and wild-type strains is estimated by plotting a group average measure of performance--for example, the mean latency to find the platform--as a function of trials. A problem with this approach is that the gradual approach to asymptote seen in group-average plots is an artifact of the averaging [1-3]. Thus, the measured quantity, the learning rate, does not reflect a meaningful quantity within the individual subjects. A second problem is that the trial-by-trial handling of the mice is stressful to them and wasteful of the experimenter's time. The results strongly reflect response to handling stress, and the paradigms cannot be scaled up to allow for large scale screening.

We believe there is a large latent demand for the large-scale behavioral screening of mutant mice for heritable malfunctions in the mechanisms of cognition. Seymour Benzer and his students, in their seminal use of genetics to get at the molecular biology of the circadian clock [4] have shown the power of this approach to take us from the behavioral to the molecular level of analysis.

We believe the keys to a successful screening program are: 1) the targeting of behaviorally well defined mechanisms, like the circadian clock, for which one can make physiologically meaningful quantitative measurements at the behavioral level (e.g., the measurement of the free-running period, or of the spectral sensitivity of its entrainment mechanism [5, 6].); 2) the development of automated procedures that eliminate handling of the mice during the period when behavioral measurements are made and give as many measurements as possible in as little time as possible.

Our research targets the interval timing mechanism, whose behavioral investigation was pioneered by Gibbon and Church [7, 8], and the mechanisms for estimating probabilities (relative frequencies) and the proportions obtaining between them. The physiologically meaningful quantities that we measure are the accuracy and precision of the individual subject's representation of these objective quantities (duration and relative frequency and proportion). We have developed

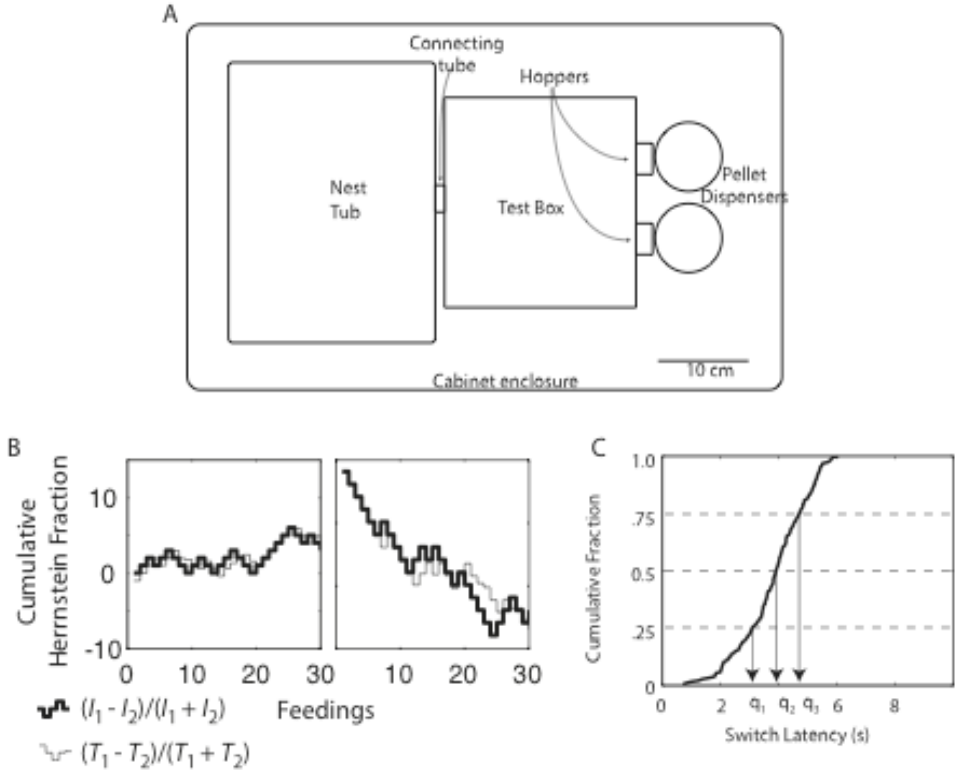

Figure 1A. *Plan of live-in test environment. A nest tub communicates with a Med Associates™ Mouse Test Box by way of a connecting tube. Test box has two illuminable feeding hoppers monitored by IR beams.* ***B.*** *Cumulative records of the Herrnstein fractions over the first 30 feedings of two experimentally naïve CB57/B6 female mice (from Gallistel et al). The Herrnstein income fraction (plotted with heavy lines) is the proportion of total pellets obtained from Hopper 1; the time fraction (light lines) is the proportion of total hopper visiting time devoted to visiting Hopper 1. When the slopes of these two cumulative records are the same, the subject is matching its visit proportion to its income proportion. (From 9)* ***C.*** *Cumulative distribution of switch latencies from a female CB57/B6 mouse, with short and long delays of 2 and 6 seconds and equal relative frequencies. The median latency (q_2) measures how accurately the subject estimates the midpoint between the delays; the inter-quartile interval ($q_3 - q_1$) measures the precision of this estimate (its variability). Balci [11] showed that the median shifts in accord with the relative frequencies of the two delays (their probability). (From 10).*

paradigms for measuring these quantities rapidly in a live-in environment, which eliminates the handling of the mice (Figure 1A). By automating every aspect of the situation, including much of the data analysis, which is conducted in quasi real time, we make it possible to do large scale screening with an equipment investment no larger than is required for many major molecular and neurobiological experimental programs.

We use the matching paradigm to measure the accuracy with which the mouse estimates the average intervals between randomly scheduled pellet releases into two different hoppers and the accuracy with which it represents the proportion between these average intervals. In the matching paradigm, the mouse adjusts the expected durations of its visits to the two hoppers so that their ratio (the proportion between the two expectations) matches the ratio of the expected intervals between pellet releases. Mice reliably exhibit matching within the first few hours in a new test environment (Figure 1B), a period during which they may remain so wary of the new environment that they eat only a few of the pellets they obtain by poking into the feeding hoppers [9].

We use the "switch" paradigm [10] to measure the accuracy and precision with which the mouse represents durations and the accuracy with which it represents a probability (relative frequency). In this paradigm, a trial begins with the illumination of the two hoppers. With some relative frequency, the trial terminates with the delivery of a pellet to, say, the left hopper after a fixed delay of, say, 2 s. With the complementary relative frequency, it terminates with the delivery of a pellet to the other hopper after a fixed delay that is longer by some fixed factor (typically in the range 1.5 to 3). The mice soon learn to begin every trial by poking repeatedly into the short-delay hopper and to switch to the long-delay hopper on those trials (long trials) when the short delay expires without the release of a pellet. The accuracy of the mouse's representation of the delays is indicated by the median of the distribution of these switch latencies; its precision by their inter-quartile interval (Figure 1C). The median switch latency also depends systematically on the relative frequency of the short and long-delay trials. It shifts toward or away from the short delay according as it is more or less probable [11].

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