Mouse lockbox: a sequential mechanical decision-making task for freely moving mice

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Abstract

Advances in automated tracking tools have sparked a growing interest in studying naturalistic behavior in animals. Yet, traditional decision-making tasks remain the norm for assessing learning behavior in neuroscience. Here, we present an alternative sequential decision-making task to study complex mouse behavior. We developed a 3D-printed mechanical puzzle, a so-called lockbox, that requires a sequence of four steps to be solved in a specific order. During the task, the mice move around freely, enabling the emergence of complex behavioral patterns. We observed that mice exhibit a high level of motivation, willingly engage in the task, and learn to solve it in only a few trials. To analyze the strategy the mice use to solve the task, we used three cameras to capture different perspectives and developed a custom data analysis pipeline. Our analyses suggest that the rapidly increasing performance is primarily due to the acquisition of manipulation skills, although first signs of a cognitive strategy for the task appear during later trials.

Keywords: freely moving mice; sequential decision making; mechanical puzzle

Introduction

Systems neuroscience research traditionally relies on relatively simple decision-making tasks. While this greatly simplifies behavioral analyses, it also constrains the rich behavioral repertoire observed in more natural settings. What is more, even simple tasks like forced two-alternative decision making tasks often require extensive training (thousands of trials) for animals like mice. In contrast, rapid learning is observed in more naturalistic contexts like fear conditioning or foraging tasks such as maze navigation (Meister, 2022).

Recent advances in automated tracking tools enable the study of behavior in more complex, freely-moving settings, but analyzing behavior without explicit task structure can be challenging. Inspired by classic puzzle boxes (Thorndike, 1911) and modern variations used in other species (Auersperg, Kacelnick, & von Bayern, 2013, Jacobson et al., 2023), we designed a novel task for mice that combines free behavior with a well-defined sequential structure. Our "lockbox" is a 3D-printed mechanical puzzle requiring mice to solve four distinct mechanisms: a lever, a stick, a ball, and a sliding door. The mechanisms need to be solved in this fixed order to access a food reward (Figure 1A). The design allows mice to observe the mechanical linkages and engage voluntarily. To analyze behavior, we recorded mice from multiple perspectives and developed an automated video analysis pipeline (Figure 1B). This pipeline uses deep learning-based pose estimation (Mathis et al, 2018, Nath et al, 2019), 3D reconstruction, and temporal filtering to classify interactions between the mouse and each lockbox mechanism, as well as the state of the mechanisms themselves.

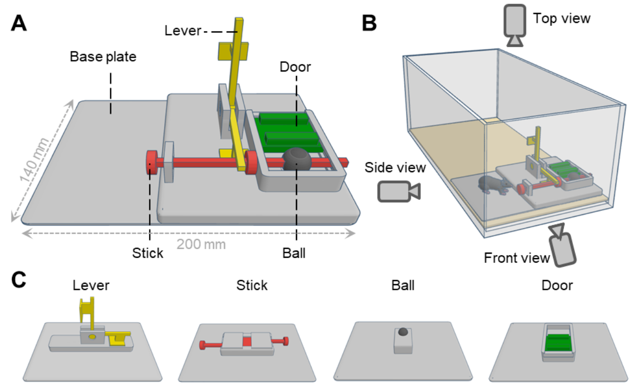

Figure 1: The lockbox task. **A,** 3D model of the combined lockbox. **B,** Schematic of the experimental setup. **C,** 3D models of the individual mechanisms, used during the single mechanism training phase.

Results

We trained a cohort of 12 mice to solve the lockbox, which were initially exposed for 30 minutes to the combined lockbox once, then underwent a single mechanism training (SMT) phase in which they were exposed to the single mechanisms (Figure 1C) in a randomized, sequential order for 11 trials. After the SMT phase, the mice were exposed to the combined lockbox for another 5 trials for a maximum of 30 minutes.

The mice readily interacted with the lockbox and demonstrated rapid learning (Figure 2A). While 3 out of 12 mice solved the task on the first exposure, 9 out of 12 succeeded in the trial immediately following the SMT phase, with success rates remaining high thereafter. This suggests that initial difficulties were partly due to needing to acquire manipulation skills for the mechanisms, which was facilitated by SMT phase where interaction times per mechanism greatly decreased (Figure 2B). The single mechanisms were consistently solved by the mice across the 11 trials with only few failure trials.

To investigate the underlying solution strategy, we discretized the behavioral data into interactions with the different mechanisms and performed a Bayesian analysis comparing a "random" interaction model (using the overall mechanism preference of a mouse as prior) against a "smart" ε-greedy model (preferring the correct mechanism for the current state). When considering the entire trial (Figure 2C, 'LSBD'), the random model provided a better fit, largely influenced by the numerous interactions during the initial 'closed' state. However, when analyzing only the later stages of the task (i.e., after the lever and stick were solved), the likelihood of the 'smart' strategy increased across trials ('BD' and 'D'), with the inferred exploration parameter ε decreasing (Figure 2D). This indicates that mice developed a solution strategy specifically for the final steps.

We further tested this by simulating 'random' agents using a Markov decision process model of the task (Figure 3A), in-

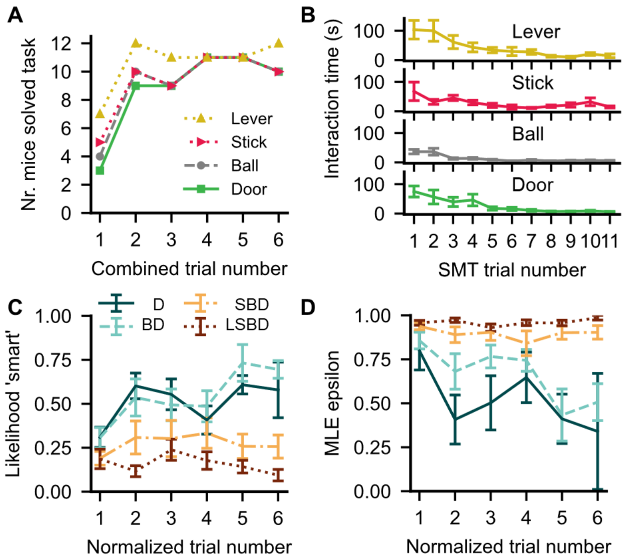

Figure 2: Mice improve through manipulation skills and strategy formation. **A,** Number of mice solving the combined lockbox. **B,** Interaction times for the SMT phase. **C,** Likelihood of mice behaving with a 'smart' (ε-greedy) strategy for various slices of the data and **D,** corresponding ε values.

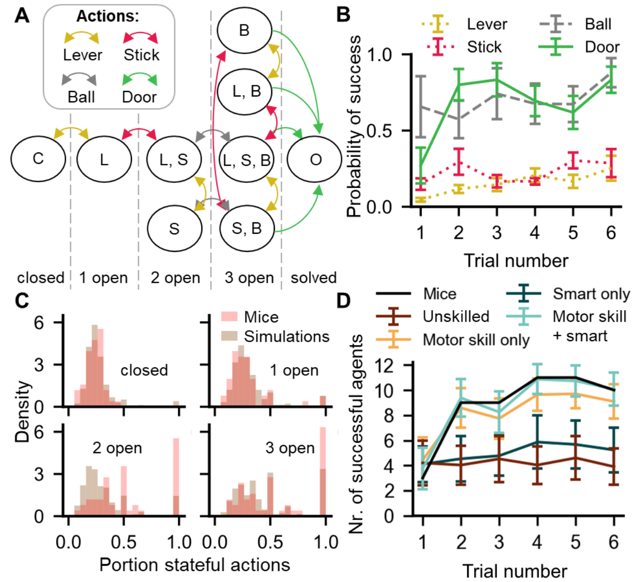

Figure 3: Simulated trial comparison. **A,** Markov state diagram representing the lockbox. Actions that do not yield state-transitions are omitted. **B,** Mouse success rates for opening lockbox mechanisms. **C,** Portion of stateful actions for the different states for mice and agents. **D,** Per-trial number of mice (or agents) solving the (simulated) lockbox task. Batches of 12 agents are sampled 100 times, having a 200-action cutoff limit per trial.

corporating overall mouse-observed mechanism preferences and mechanism opening/closing success rates (Figure 3B). While the overall solving portion and average actions could be matched, the distribution of 'stateful' actions (interactions with the currently correct mechanism for a given state) differed between mice and random agents in the later '2 open' (ball) and '3 open' (door) states (Figure 3C). Mice performed stateful actions more often than predicted by the random model in these later states, supporting the Bayesian analysis finding of a developing strategy.

To delineate different types of learning (i.e., the acquisition of manipulation skills to open the mechanisms and a higher-level cognitive strategy), we simulated each possible combination in a separate model and qualitatively compared their performance with that of the mice (Figure 3D). The 'unskilled' agents adopt a random (i.e., state-independent) strategy, and their state-transition probabilities are fixed to the mouse success rates from the first trial in Figure 3B. The 'motor skill only' agents have dynamic per-trial state-transition probabilities, corresponding to the success rates of mice across trials. The 'smart only' agents have the same state-transition probabilities as the 'unskilled' agents, but adopt an ε-greedy policy for the later states, based on the Bayesian inference results and the number of successful mice. Lastly, the 'motor skill + smart' agents contain both features. We find that, while the inclusion of skill learning is the largest contributor to match the mouse learning curve, the combination of developing object manipulation skills and a higher-level task strategy is necessary to fully recreate the mouse learning behavior.

Conclusions

We introduced the lockbox as a novel tool for studying sequential decision-making and mechanical problem-solving in freely moving mice. Mice learned this relatively complex, four-step task surprisingly quickly compared to conventional operant tasks, demonstrating its potential utility of more ethologically relevant challenges. Our analysis pipeline allowed detailed quantification of behavior, revealing that learning involved both rapid improvement in object manipulation skills and the emergence of a task-specific strategy. Interestingly, this strategic improvement appeared predominantly in the later stages of the sequence, suggesting a possible shift from exploration to exploitation within a trial as the goal nears.

The fact that mice didn't immediately adopt a strategy, relying mostly on random exploration biased by preference (especially initially), could reflect an ecologically sound approach. In environments where problems vary, investing heavily in learning specific sequences may be less beneficial than developing generalizable motor skills and effective exploratory heuristics. The lockbox paradigm, by requiring distinct manipulations, allows dissecting these components. Future studies could explore generalization by altering the sequence or mechanisms or utilize automated setups for longitudinal studies with larger datasets to track the fine-grained evolution of skill and strategy over extended periods.

Acknowledgements

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2002/1 "Science of Intelligence" – project number 390523135.

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