Y-maze and Vertical Pole Performance Correlate with Fine Motor Learning in Mice

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Abstract

When a new memory is consolidated, it can leave behind a residual trace that affects the formation of subsequent memories for similar tasks. However, the relationships between distinct abilities and their interdependencies during memory tasks and motor learning activities are not clear. An important question is whether being proficient in memory or motor learning tasks also translates into better performance in another, similar task - reflecting potential generalization of motor learning abilities. To investigate the correlation between memory performance and motor learning in mice, we used a combination of behavioral tasks that assess general exploratory behavior, declarative memory, and fine motor learning. For the exploratory behavior, we used the Open Field task, and assessed declarative memory using the Novel Object Recognition and Y-maze. Motor learning was assessed through the Vertical Pole and Pellet Reaching task. We found a negative correlation between the Vertical Pole task and the motor learning task, where a shorter time for an individual to turn on a pole correlated with an increased number of attempts to reach a sugar pellet in the motor learning task. Additionally, a positive correlation was found between the Y-maze and the motor learning task, where a higher exploration rate indicated a higher success ratio in the pellet reaching task. These results can be used to conduct a pre-study for challenging motor tasks that include pre-behavioral procedures on mice. Our study indicates that both the Vertical Pole and the Y-maze can be suitable predictors of motor learning performance and activity.

Keywords: Memory, Motor learning, Behavioral tasks

Introduction

Memory consolidation has long been recognized as a critical component of learning and adaptation [1, 2]. However, the intricate relationships between distinct cognitive abilities and their interdependencies during memory tasks and motor learning activities remain poorly understood. Motor learning is a function to refine skilled movements through practice [3], which allows for adjustments of voluntary movements. This is a key function for adaptive behavior during exploration and learning, as the movements can be performed with higher speed and accuracy [4, 5]. Motor learning involves activity across multiple interconnected brain regions, including the motor cortex, basal ganglia and cerebellum [6, 7, 8].

Interestingly, neural circuits underlying previously learned tasks can be repurposed and modified to accommodate new learning, while retaining their existing memory traces [9]. This phenomenon suggests that memories from one task are not only dynamic but also context-dependent, allowing them to coexist with and influence the formation of novel memories. Specifically, experiences can serve as a foundation for subsequent learning, enabling individuals to leverage past knowledge to inform and improve their performance on novel tasks [9]. Furthermore, findings from motor learning studies have consistently demonstrated individual variability in the acquisition and refinement of controlled voluntary movements [10], highlighting the complex interplay between cognitive and motor processes.

Although the biological mechanisms of episodic memory and motor learning have been extensively studied, there is a gap in knowledge regarding the correlation between individual differences in these functions. Previous research has analyzed some connection between behavior tasks, although the focus was to investigate the effect an agent had on memory, not the direct correlation. A study from Kelliny et al. [11] examined the effects of streptozotocin (STZ), a diabetogenic agent, on mice behavior, and found that STZ-treated mice showed anxiety-like behavior and reduced exploratory behavior in the open field task, and simultaneously performed worse in declarative memory tasks, by investigating spontaneous alternation in the Y-maze task and recognition memory in the novel object recognition task [11]. These tasks were performed in parallel, but the study did not assess the direct correlation between them. However, the results suggest that it might be possible to predict individual performances in behavior tasks based on results from others. To address this knowledge gap, we employed a combination of

behavioral tasks that assess general exploratory behavior, declarative memory, and fine motor learning in mice, allowing investigation of correlations between these distinct cognitive and motor abilities.

A challenge in performing animal studies where the subjects must execute a task that requires learning is the fact that the process of learning is highly individual. When the mechanisms of learning are not the study's focus, it is important to reduce the experimental variance caused by the individual's ability to learn a task. In addition, by identifying and selecting animals performing well in the task, the likelihood of successful future experiments involving more invasive procedures, such as optic ferrule implantations (optogenetics) and lens implants (miniscope) increases, by decreasing the likelihood of performing pre-behavioral procedures, likely chronic implants on mice that may not perform well. An idea to achieve this is to perform a selective study to identify individuals with a higher likelihood to learn a challenging task based on tasks involving memory and motor execution in naïve mice, where individuals performing well in the tasks are selected for further investigation. This study aims to find a possible predictor task for improved performance in a challenging motor task – the pellet reaching task. Behavior tasks selected are open field, novel object recognition, Y-maze and vertical pole, due to their simpler and shorter execution and interpretation. The hypothesis is that mice that perform well in general behavior tasks that include motor coordination will also perform well in the pellet reaching task.

Methods

Animals

All experiments were performed with the ethical permit (number 5.8.18-07526/2023) approved by the Swedish Board of Agriculture. The animals used in this study are heterozygous mice from the Chrna2-cre line crossed with C57Bl/6J wild-type mice. Twelve mice, aged four months at the beginning of the experiments, were housed in groups of two to four individuals per cage in a temperature-controlled room with a 12-hour cycle of daylight and darkness, with access to water ad libitum. Cages were lined with wood chip bedding and enriched with paper houses and nesting materials. Food was available ad libitum, unless on occasions when the animals were food-restricted. In those cases, the restriction lasted a maximum of 20 hours. All mice used in this study were female and kept at a body weight of a minimum 90% of their starting body weight. In the open field test, all 12 mice were included. In the novel object recognition task, nine mice were included, and three mice were temporarily excluded due to an unstable object movement during recording. In the Y-maze and the vertical pole task, all 12 mice were included. Due to an observed illness, one of the mice was excluded from the pellet reaching task, and 11 were included.

Habituation

The habituation procedure was conducted over the course of five consecutive days. The animals were brought to the behavior room to acclimate for 30 minutes. To minimize stress, the exposure to humans was gradually increased. The room's light intensity was gradually increased to goal-light day by day, spanning from five lux initially to 80 lux at the end of the habituation process, which was the same light intensity used in the following tasks. The duration of each habituation session was five minutes, performed twice a day with a minimum of two hours of rest between the sessions. Before each task, the animal home cages were brought to the behavioral room to acclimatize (15-30 minutes before initiating the task). For each task, mice were placed individually in the arena. To minimize stress levels in the task, we checked the animal's identity after the trials.

Open field

The Open-Field test is used in this study to assess locomotion and exploratory behaviors when animals are exposed to an empty arena. The task requires little animal training, and only one test session is needed. This task was mainly used to habituate the animals to the arena before novel object recognition, and to measure a baseline for general exploratory behaviors. To assess locomotion and to habituate mice to the arena, individual mice were placed in an empty round arena, measuring 46 cm in diameter. For easy detection by the recording device, the chosen arena was white. The recording time was five minutes, and one session was recorded for each mouse. After five minutes, the animal was brought to a new cage, and the arena was cleaned thoroughly. The behavior was recorded with a JVC camera. The extracted measurements were distance moved, velocity and time spent in the center.

Novel object recognition

This task was performed over the span of two days [using the same parameters, settings and setup as previously described; 12]. The experiment set-up consisted of a round

46 cm diameter white arena (same as open field), two identical "familiar" objects, one "novel" object, a JVC camera for recording the material, and a computer. Two similar dish brushes that had been modified and attached to a lid, to allow them to stand upright, were used as familiar objects. The novel object was a dish brush with a different shape. For testing, animals were brought to the arena one by one and spent 10 minutes exploring the objects. After 10 minutes, the animals were brought to a new cage, and the arena was thoroughly cleaned before bringing the next animal. The objects were cleaned with 10% ethanol and a paper tissue while still being placed in the arena, to ensure that the objects are in the same place for each animal.

On the first test day, the animals were exposed to two objects. The next test day, one of the objects was replaced with a novel object. The novel object was similar to the familiar objects in terms of shape, size, and color, but still easily distinguishable. Both days were recorded using a JVC camera mounted directly above the arena. For analysis, the time spent investigating the familiar vs novel object on test day was calculated. The ratio was calculated as the time spent investigating the novel object divided by the sum of the times investigating the familiar and novel object, then converted to percentage by multiplying by 100.

Y-maze

The Y-maze assesses short-term spatial working (declarative) memory in mice, driven by their innate curiosity and motivation to explore new areas [13]. The task is carried out by allowing the animal to freely explore a Y-shaped arena (L: 53.5 cm; W: 5 cm; H: 10 cm) while tracking the spontaneous alternation of entries into each arm. A continuous pattern of entry into each arm characterizes an alternation. The number of alternations is the results that indicate a good or bad working memory. For example, if the animal has explored the maze by entering arm A and then arm B, the next arm would be arm C. If the animal has a low number of correct alternations, their working memory might be impaired.

For testing, the arena was elevated on the floor to minimize the risk of escapes, built directly beneath the JVC camera. The animals were brought to the behavior room and left for 30 minutes to acclimate, and then brought to the arena one by one and placed in arm B to explore the maze alone for 10 minutes. Analysis was made by calculating the number of correct alterations divided by the total number of alternations, multiplied by 100.

Vertical pole

The principle of the vertical pole task is to assess fine motor coordination skills in mice [14]. A 50 cm high metal pole was used, covered in masking tape to make ridges that the mice can grab onto. The mice were placed at the top with their face turned upwards. The measurements for the task are the time to turn and time to descend. The time to turn describes the time it takes for the mouse to turn from being placed on top of the pole, face up, until it has turned its body 180° in alignment with the pole. The time to descend measures the time for the mouse from being placed on the pole, faced up, until it has reached the bottom of the pole [14].

Before training and testing, the animals were allowed to acclimate in the room for 10 minutes. The pole was placed in an empty cage on a table in the experimental room. The animals were trained for two days before the task, and during these sessions, they were habituated to the test setting. Each animal was trained to climb the pole by being placed halfway up, faced down, and allowed to climb down the pole with support from the experimenter as needed to reduce the risk of falling. When the animal had managed to climb down half the pole, it was instead placed at the top of the pole, faced down, and allowed to climb down freely with support if needed. After successful climbs, the mouse was placed an additional time at the top of the pole, this time faced up, to simulate the real test setting and allowed to climb down freely. This training trial was repeated until the animal was able to climb down securely. In between the training trials, the animal was left to roam the cage for 30 s.

Before the test session, a video camera (Canon Legria HF M52 10x optical zoom) was mounted on a stand and directed towards the pole, with both the top and bottom in frame. The task was carried out with three consecutive test trials, with 30 s of free roam time in the experiment cage between trials.

Pellet reaching

The pellet reaching task is a widely used task to assess fine motor learning and skilled forelimb use in rodents [15]. The mice were trained and habituated to the arena for four consecutive days. Both training sessions and test sessions lasted for 10 minutes. The first two days, mice were placed in the arena in groups of two to three individuals together. Sugar pellets (Non paraille, Dr. Oötger) were sprinkled on the floor of the arena, and the animals were allowed to explore the arena while the experimenter stayed close to make the animals used to human contact.

In the third and fourth session, the animals were trained one by one, and the pellets were offered on a metal spoon through the slit instead of sprinkled on the floor.

During the test sessions, the animals were tested one by one and were allowed to take a pellet only from the spoon in a ten-minute session. For each test session, the animals were food restricted to enhance their motivation to reach for the sugar pellets. Before removing the food, each animal was weighed to verify whether they had lost more than 10% of their initial body weight. All animals used in this study maintained at least 90% of their body weight throughout the experiments. Food was removed 15 hours before the experiment was initiated and put back after no more than 20 hours. Three test days were executed, with a minimum of one rest day in between.

The task was recorded with a video camera (Canon Legria HF M52 10x optical zoom) mounted on a tripod, with the arena slit in frame. The trials were categorized into successful or failed reaches. A successful reach occurred when the animal could effectively grab the pellet and eat it. A failed reach occurred when the animal could not complete the task, either by missing, hitting, and dropping the pellet, grasping and dropping before eating, or reaching for the pellet when no pellet was available.

Statistical analysis

The effect of object familiarity on novel object recognition and the effect of behavioral arena on total distance moved were analyzed using one-way repeated measures ANOVA, followed by paired t-tests for post-hoc pairwise comparisons. Due to the low number of data points, correlation statistics were performed using the Spearman coefficient with p-values calculated via permutation, to assess the relationships between each behavioral measure and the motor learning task. r values close to +1 measure positive correlation, meaning when one variable increases, the other variable increases linearly. Values close to 0 mean there is no correlation, and values close to -1 indicate a negative correlation, meaning when one variable increases, the other variable decreases proportionally. To evaluate the triple correlation among three variables, we first computed the multiple correlation coefficient based on their pairwise correlations, representing the shared linear variance. This coefficient was then adjusted for sample size and the number of variables to obtain the multiple coefficient of determination. An F-statistic was subsequently calculated from the adjusted correlation and degrees of freedom to test the significance of the observed relationship, with the corresponding p-value derived to assess statistical significance.

Software availability

Video recordings for the open field, novel object recognition, and Y-maze tasks were obtained using EthoVision software version 15, and behavioral data tracking and extraction were performed using EthoVision software version 16. Data analysis was conducted using Scipy [16], Numpy [17] and SciScripts [18], while all plots were generated with Matplotlib [19]. Schematics were created using Inkscape [20]. The data analysis script is available online [21].

Results

Mice were tested in the open field, novel object recognition, Y-maze, vertical pole, and pellet reaching tasks to examine whether refined motor learning skills correlate with more generalized motor performance (Figure 1A). To assess this, multiple measures were extracted from each task, including the locomotion in the open field, as a baseline for how much the individuals tend to move in the arenas (Figure 1B-K). As expected due to the intrinsic drive to explore a new object compared to a known object [22], the task (Figure 1E-F) showed that most individuals explored the new object more than the familiar object (8 out of 9 Mean investigation time for the new object was 88.94 s vs 47.29 s for the familiar object; Figure 1E). This shows that the animals could remember which object was replaced, and their innate curiosity motivated them to investigate the new object over the familiar one during the testing session. The ratio showed that individuals spent on average double the time examining the new object than the familiar one (Figure 1F). These results indicate that the group has good recognition memory.

To assess working memory, we used the Y-maze task. Correct alternation suggests good working memory, and the mean value for the correct alternation ratio for the group was 68.5% (Figure 1I). The vertical pole task was used to evaluate motor coordination by measuring the mean time for animals to turn and descend from the pole (Figure 1L-M). The group mean time for the time to turn was 1.7 s, and for the time to descend, the mean time was 6.2 s. In the pellet reaching task, we evaluated how well the animals can learn a complex motor task. The mean attempts of reaches in the group was 176 times (Figure 1N), and of those, the mean value of successful reaches was 65 times (Figure 1O), giving the group a success ratio of 35% (Figure 1P). This ratio can be interpreted as the individuals in general are active and reach for the pellets, but only a third of the time succeed in grabbing them.

The distance moved and velocity were evaluated in the open field, novel object recognition, and y-maze arenas

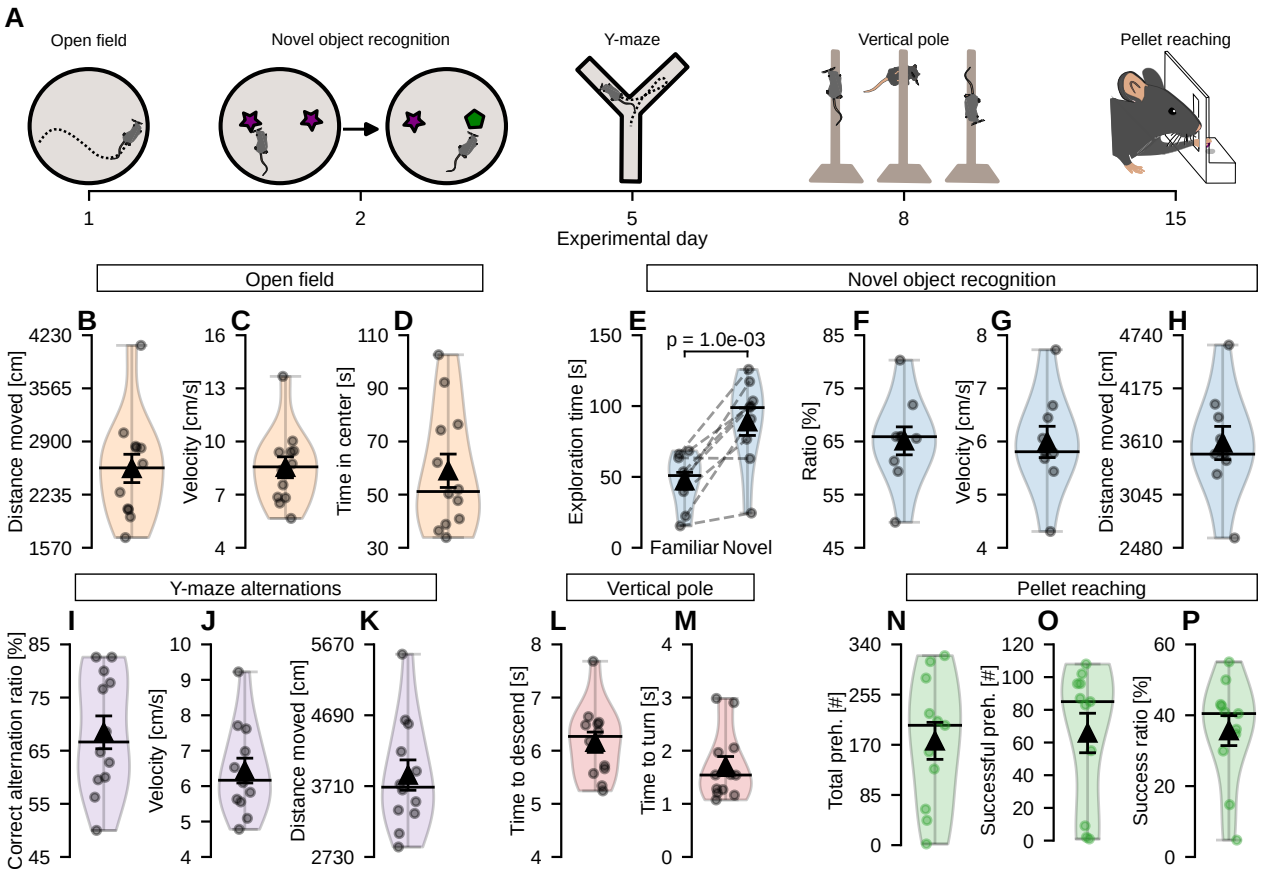

Figure 1: Mice performance in each behavioral task. A) Timeline of experiments, with experimental days where the tasks were initiated. B-D) Distance moved (B), Velocity (C) and time spent in the center of the arena (D) during the open field test. E) Exploration time for familiar versus novel objects. F) Ratio of exploration of the familiar in relation to the novel objects. G-H) Velocity (G) and Distance moved (H) during the novel object recognition task. I-K) Ratio of correct alternations (I), Velocity (J), and distance moved (K) during the Y-maze task. L-M) Time to descend from the top of the pole to the floor (L), and time to turn on the top of the pole faced up until the animal is completely aligned with the pole faced down (M) in the vertical pole task. Each datapoint is the mean value from 3 consecutive trials for each mouse. N-P) Total number of reaches (N), total number of successful reaches (O) and success ratio measured in percentage (P) for the pellet reaching task. The horizontal lines show the medians, and triangles show the means.

to verify whether there are animals spending most of the time standing still in one of the tasks. Comparison of the measurements in each task (novel object recognition and Y-maze) with open field test measurements indicated one outlier that travelled further distance and faster average velocity compared to the rest of the group (4102.7 cm traveled distance at 13.68 cm/s vs group mean 2709.46 cm traveled at 9.03 cm/s in the open field test), but no outliers that travelled less distance or at slower velocity compared to the group mean (Figure 2A-B). Also, comparing the distance moved and velocity in Y-maze and open field shows that all individuals travel a greater distance during the Y-maze task, but at slower speeds, which indicates that the response to the novel arena was not immobility (Figure 2A-B). This comparison verifies that the results from the Y-maze task are not influenced by stress or anxiety due to handling, solely exploring the maze at the same rate as exploring the empty arena.

After extracting measurements from all tasks, we investigated whether they are correlated, which would indicate that skills to perform correlated tasks could be similar or predict one another. We first investigated if there is any correlation between the open field and novel object recognition, which are exploratory, with the y-maze, which specifically tests working memory. The correlation analysis between the Y-maze measurements and the novel object recognition and open field tests indicated no significant correlation, suggesting that the mice's performance in either novel object recognition or open field can predict the performance in the Y-maze (Figure 2C).

Correlation analysis between each measure from the behavior tasks and the vertical pole task indicated significant correlation between the traveled distance in novel object recognition and the two measures time to descend and time to turn in the vertical pole task (Figure 2D-2F). A greater traveled distance in the novel object recognition

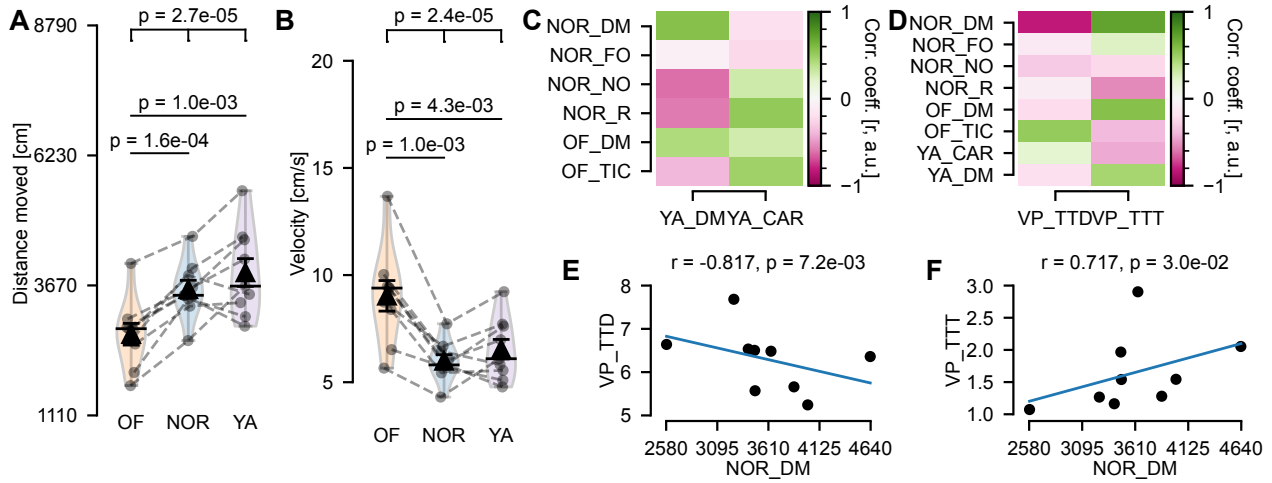

Figure 2: Mice with faster descending times in the vertical pole task travelled larger distances in the novel object recognition task. A-B) Distance moved (A) and velocity (B) in the open field, novel object recognition and Y-maze. C) Correlation matrix between novel object recognition and y-maze measures. D) Correlation matrix of measures from novel object recognition, open field, and Y-maze versus vertical pole. E-F) Correlation observed between the traveled distance in the novel object recognition task and time to descend (E) and time to turn (F) in the vertical pole task. Abbreviations: NOR (Novel Object Recognition); OF (Open Field); VP (Vertical Pole); YA (Y-maze); DM (Distance Moved); FO (Familiar Object); NO (Novel Object); R (Ratio); TIC (Time In Center); TTD (Time To Descend); TTT (Time To Turn); CAR (Correct Alternation Ratio).

task correlated with a quicker descent (r=-0.817, p=7.2e-03; Figure 2E), and a longer time to turn (r=0.717, p = 3.0e-02; Figure 2F) in the vertical pole task. This finding suggests that animals with a greater traveled distance in the novel object recognition task will perform well in the descent, but at the same time take longer to turn.

When evaluating the correlation between all measurements from all behavioral tasks and the pellet reaching task measures, we found correlations between five pairs of measures (Figure 3A). A positive correlation is exhibited between the time spent in center in the open field test and the number of attempts to grab pellets in the pellet reaching task (r=0.664, p = 2.6e-02; Figure 3B). A negative correlation can be seen between the time to turn variable in the vertical pole, and the sum of successful reaches in the pellet reaching task (r=-0.624; p = 4.0e-02; Figure 3C). The same measure also correlated negatively with the sum of failed attempts (r=0.645, p = 3.2e-02; Figure 3D) and the sum of all reaches (r=-0.627, p = 3.9e-02; Figure 3E). This indicates that an individual who turns faster (displaying better paw coordination) is more likely to try and reach for pellets than an individual who turns slower. Additionally, the analysis displayed a positive correlation between the pellet reaching success ratio and the Y-maze correct alternation ratio. Individuals who had the correct pattern when making a turn in the Y-maze (indicating better working memory) had a higher success ratio when reaching for sugar pellets (r=0.788, p = 4.0e-03; Figure 3F). Interestingly, when investigating a triple correlation, with the vertical pole time to turn and the Y-maze correct alternation ratio as independent variables and the pellet reaching success ratio as the dependent variable, we found a strong correlation between the pellet reaching success ratio and both independent variables (r = 0.794, p = 1.9e-02; Figure 3G). This suggests that animals with higher performance in both the vertical pole and Y-maze tasks are more likely to succeed in the pellet reaching task, indicating a potential link between these motor and memory-related behaviors and motor learning outcomes.

Discussion

This study aimed to identify whether behavioral performance in memory and motor tasks correlates with subsequent motor learning success. By examining correlations between tasks such as the Y-maze and pellet reaching, we investigated potential predictions that can be useful in future studies that require high motor learning capacity. Results showed significant correlations between the time to turn in the vertical pole task and the total number of reaches, total number of failed reaches, and total number of successful reaches in the pellet reaching task. The correlation was negative because the time to turn is inversely related to performance - shorter times indicate better motor coordination, therefore animals that turn more quickly on the vertical pole were more likely to attempt to reach and grab the pellet. These results suggest that performance in the vertical pole task might predict the level of activity in motor tasks, such as pellet reaching, and the success rate in the task. These findings can be useful in

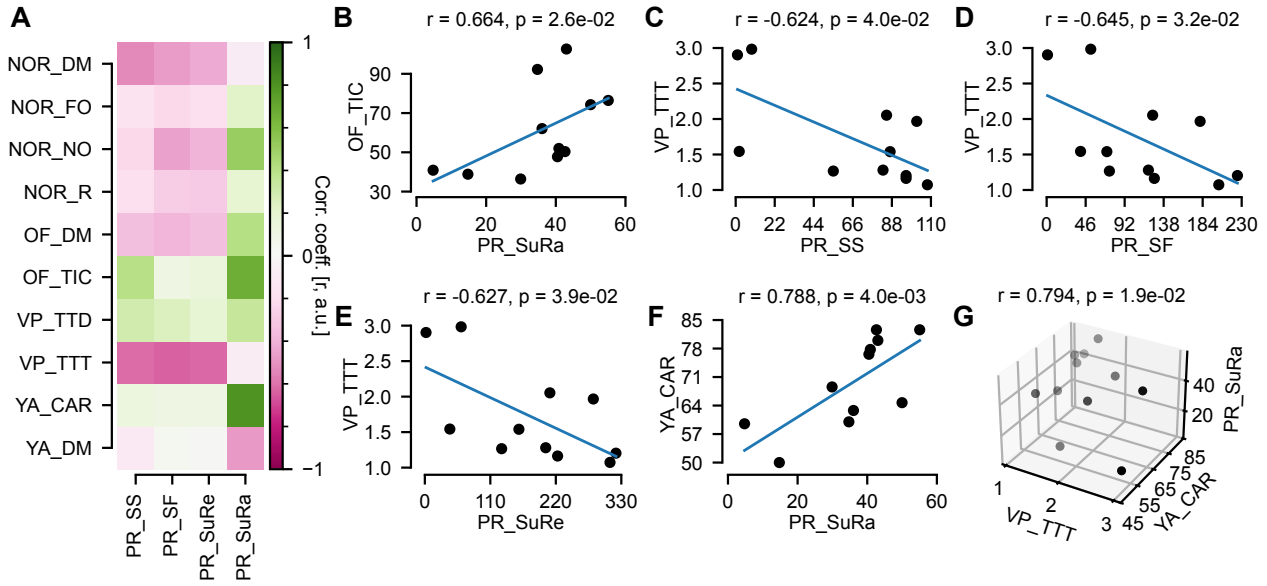

Figure 3: Vertical pole and Y-Maze measures correlate with pellet reaching measures. A) Correlation matrix between each task measure versus the pellet reaching task measures. B) Open field time in center showed a positive correlation with the pellet reaching success ratio. C-E) Vertical pole time to turn showed a negative correlation with the sum of successful (C) and failed (D) reaches, and with the sum of all reaches (E). F) Y-maze correct alternations showed a positive correlation with the success ratio. G) Triple correlation showing that success ratio is correlated to both vertical pole time to turn and Y-maze correct alternations. Abbreviations: NOR (Novel Object Recognition); OF (Open Field); VP (Vertical Pole); YA (Y-maze); DM (Distance Moved); FO (Familiar Object); NO (Novel Object); R (Ratio); TIC (Time In Center); TTD (Time To Descend); TTT (Time To Turn); CAR (Correct Alternation Ratio); PR (Pellet Reaching); SS (Sum of Success); SF (Sum of Fail); SuRe (Sum of all Reaches); SuRa (Success Ratio).

future studies, as the performance in the time to turn in the vertical pole task can predict both how active they are in the pellet reaching task and how well they learn the movement to grab pellets. Interestingly, the time to descend showed no correlation with pellet reaching, showing that the turning part of the task is more closely related to motor learning than the descending part. While turning, the mouse is required to adjust and correct movements without falling.

It is unclear whether a task can both predict and improve subsequent motor behavior. A study examined how skills learned in one task can be transferred to another. Participants learned three different motor tasks in a row, each with varying levels of feedback. The results showed that learning one task made it easier to learn the next ones, and that the order in which they were learned affected how well transfer was achieved [23]. As both vertical pole turning and grabbing small sugar pellets include motor precision, the vertical pole task might be a proper choice to consider to predict fine motor skills behavior in the pellet reaching task. However, the descent in the vertical pole task did not show any significant correlation with the pellet reaching task. Descending the pole might require less fine motor coordination, and a possible fear of heights might also be reflected in the time spent climbing down. The innate fear of heights could affect the animal's motivation to climb down the pole or cause hesitation in the movements. Similar responses can be observed in other behavioral tasks that include high altitudes, such as the elevated maze task, which depends on the mice's aversion to heights [24]. This observation could explain why the descend performance does not reflect performance in the pellet reaching task. In turn, our results suggest that the turning movement is more closely related to fine motor skills than climbing is.

The results from analyzing the correlation between the vertical pole task and pellet reaching task are more likely to indicate higher activity levels than motor learning, since we observed differences in the sum of success, fail and total attempts but not in the success ratio. While the results did not show a direct correlation with the success ratio between these tasks, higher activity levels may in turn lead to a higher learning rate. Although this explanation is yet to be studied directly, a similar mechanism was observed in a study where young adults with higher physical activity levels exhibited faster reaction times, indicating that increased activity correlates with enhanced cognitive and motor performance [25].

While the vertical pole task correlated with the number of reaches and number of successful reaches in the pellet reaching task, the Y-maze task correlated with the ratio of successful reaches. This suggests that the

Table 1: Table of all possible correlations found in the tasks. The correlating measures are marked in highlights. Abbreviations: NOR (Novel Object Recognition); OF (Open Field); PR (Pellet Reaching); VP (Vertical Pole); YA (Y-maze); FO (Familiar Object); NO (Novel Object); R (Ratio); DM (Distance Moved); TIC (Time In Center); SS (Sum of Success); SF (Sum of Fail); SuRe (Sum of all Reaches); SuRa (Success Ratio); TTD (Time To Descend); TTT (Time To Turn); CAR (Correct Alternation Ratio).

	NOR_FO	NOR_NO	NOR_R	NOR_DM	OF_DM	OF_TIC	PR_SS	PR_SF	PR_SuRe	PR_SuRa	VP_TTD	VP_TTT	YA_CAR	YA_DM
NOR_FO	r = 1.0 p = 0.0													
NOR_NO	r = 0.533 p = 0.139	r = 1.0 p = 0.0												
NOR_R	r = -0.483 p = 0.187	r = 0.333 p = 0.381	r = 1.0 p = 0.0											
NOR_DM	r = 0.0 p = 1.0	r = -0.05 p = 0.898	r = -0.167 p = 0.668	r = 1.0 p = 0.0										
OF_DM	r = 0.0 p = 1.0	r = -0.233 p = 0.546	r = -0.35 p = 0.356	r = 0.717 p = 3.0e-02	r = 1.0 p = 0.0									
OF_TIC	r = 0.367 p = 0.332	r = 0.383 p = 0.308	r = -0.15 p = 0.7	r = -0.65 p = 0.058	r = 7.0e-03 p = 0.983	r = 1.0 p = 0.0								
PR_SS	r = -0.167 p = 0.668	r = -0.233 p = 0.546	r = -0.2 p = 0.606	r = -0.533 p = 0.139	r = -0.355 p = 0.284	r = 0.392 p = 0.233	r = 1.0 p = 0.0							
PR_SF	r = -0.233 p = 0.546	r = -0.467 p = 0.205	r = -0.283 p = 0.46	r = -0.483 p = 0.187	r = -0.391 p = 0.235	r = 0.1 p = 0.77	r = 0.907 p = 1.2e-04	r = 1.0 p = 0.0						
PR_SuRe	r = -0.2 p = 0.606	r = -0.4 p = 0.286	r = -0.3 p = 0.433	r = -0.433 p = 0.244	r = -0.355 p = 0.285	r = 0.136 p = 0.689	r = 0.934 p = 2.6e-05	r = 0.991 p = 3.8e-09	r = 1.0 p = 0.0					
PR_SuRa	r = 0.217 p = 0.576	r = 0.5 p = 0.17	r = 0.183 p = 0.637	r = -0.1 p = 0.798	r = 0.4 p = 0.223	r = 0.664 p = 2.6e-02	r = -0.018 p = 0.958	r = -0.182 p = 0.593	r = -0.155 p = 0.65	r = 1.0 p = 0.0				
VP_TTD	r = -0.117 p = 0.765	r = -0.3 p = 0.433	r = -0.083 p = 0.831	r = -0.817 p = 7.2e-03	r = -0.217 p = 0.499	r = 0.517 p = 0.085	r = 0.296 p = 0.377	r = 0.245 p = 0.467	r = 0.173 p = 0.612	r = 0.336 p = 0.312	r = 1.0 p = 0.0			
VP_TTT	r = 0.233 p = 0.546	r = -0.233 p = 0.546	r = -0.533 p = 0.139	r = 0.717 p = 3.0e-02	r = 0.566 p = 0.055	r = -0.378 p = 0.226	r = -0.624 p = 4.0e-02	r = -0.645 p = 3.2e-02	r = -0.627 p = 3.9e-02	r = -0.082 p = 0.811	r = -0.182 p = 0.572	r = 1.0 p = 0.0		
YA_CAR	r = -0.234 p = 0.544	r = 0.301 p = 0.431	r = 0.527 p = 0.145	r = -0.192 p = 0.62	r = 0.294 p = 0.353	r = 0.483 p = 0.111	r = 0.128 p = 0.708	r = 0.105 p = 0.759	r = 0.105 p = 0.759	r = 0.788 p = 4.0e-03	r = 0.193 p = 0.549	r = -0.434 p = 0.158	r = 1.0 p = 0.0	
YA_DM	r = -0.067 p = 0.865	r = -0.617 p = 0.077	r = -0.583 p = 0.099	r = 0.567 p = 0.112	r = 0.427 p = 0.167	r = -0.385 p = 0.217	r = -0.123 p = 0.719	r = 3.6e-02 p = 0.915	r = 1.8e-02 p = 0.958	r = -0.491 p = 0.125	r = -0.189 p = 0.557	r = 0.455 p = 0.138	r = -0.49 p = 0.106	r = 1.0 p = 0.0

Y-maze might be a more accurate choice than the vertical pole task for selecting candidates for a motor task study. However, it is important to note that the success ratio is not the only variable to consider when analyzing the results. For example, an individual who only tried to reach for a pellet twice and succeeded once would have a 50% success ratio. The inactivity or disinterest in the task makes it difficult to analyze the learning capacity of the individual if the only measure is the success ratio; hence, the total number of reaches is important. Also, the triple correlation analysis revealed that the pellet reaching success ratio is correlated to both the vertical pole time to turn and the Y-maze correct alternation ratio, showing that including both the Y-maze and vertical pole could provide a more comprehensive assessment of a mouse's likelihood to learn, as together they may identify individuals with higher engagement and learning potential. Interestingly, we found no correlation between any of the vertical pole or Y-maze measures, suggesting that while increased performance on both tasks is associated with increased fine motor coordination (pellet reaching task), gross motor coordination (vertical pole) is not directly linked to working memory (Y-maze).

Another intriguing finding reveals that animals that excelled in the pellet reaching task showed reduced stress levels by spending more time in the center of the open field arena, suggesting a correlation between decreasing anxiety and improved motor performance. Similarly to what is described in the literature where increased anxiety levels can have a negative impact on different perceptual-motor tasks [26]. Likewise, in the vertical pole, animals that spent less time descending from the pole tended to cover longer distances in the object recognition task, revealing an association between increased exploratory behavior, and motor coordination. Interestingly, no other behavior measure correlated with the pellet reaching task (Table 1, Supplementary Figure 1).

Results from this study are significant for future studies, especially behavioral studies using optogenetics or miniscopes, which involve invasive surgery that heavily rely on animals that can learn a task. Using a non-invasive test as a prediction enables researchers to only include animals likely to learn while minimizing the harm to non-learning mice. These results are particularly important for motor tasks like the pellet-reaching task, which are time-consuming and involve food deprivation, potentially causing distress to the animals involved. A pre-selection process can therefore increase the effectiveness of the following behavior studies, and at the same time, adhere to the principles of the 3 Rs - replacement, reduction, and refinement by reducing the number of animals used in the experiments, as non-learners are not included.

In summary, the Y-maze task is useful as a predictor for motor learning tasks. Additionally, the time to turn in the vertical pole task correlated with a higher rate of reaches in total. This aligns with the hypothesis that performance in tasks involving motor functions correlate with performance in the pellet reaching task. We conclude that the Y-maze and vertical pole tasks are suitable

to pre-select good candidates for behavioral studies that include motor skill acquisition.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

Methodology (IK, SS, BC), Data curation (IK, SS, BC), Validation (SS, BC), Formal analysis (TM), Visualization (IK, TM, BC), Original draft preparation (IK, BC), Conceptualization (KK, SS, BC), Writing - Reviewing and editing (IK, TM, KK, SS, BC).

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Data Availability Statement

The datasets generated and/or analyzed in the current study are available on request.

References

[1] Yadin Dudai, Avi Karni, and Jan Born. The consolidation and transformation of memory. *Neuron*, 88(1):20â€“32, October 2015. ISSN 0896-6273. doi: 10.1016/j.neuron.2015.09.004. URL http://dx.doi.org/10.1016/j.neuron.2015.09.004.

[2] Emily T. Cowan, Anna C. Schapiro, Joseph E. Dunsmoor, and Vishnu P. Murty. Memory consolidation as an adaptive process. *Psychonomic Bulletin & Review*, 28(6):1796â€“1810, July 2021. ISSN 1531-5320. doi: 10.3758/s13423-021-01978-x. URL http://dx.doi.org/10.3758/s13423-021-01978-x.

[3] Cathrine V. Jansson-Boyd and Peter Bright. *Memory and learning*, page 93â€“118. Elsevier, 2024. ISBN 9780443135811. doi: 10.1016/b978-0-443-13581-1.00006-6. URL http://dx.doi.org/10.1016/b978-0-443-13581-1.00006-6.

[4] Daniel B. Willingham. A neuropsychological theory of motor skill learning. *Psychological Review*, 105(3):558–584, 1998. doi: 10.1037//0033-295x.105.3.558. URL https://doi.org/10.1037//0033-295x.105.3.558.

[5] Eran Dayan and Leonardo G. Cohen. Neuroplasticity subserving motor skill learning. *Neuron*, 72(3):443–454, November 2011. ISSN 0896-6273. doi: 10.1016/j.neuron.2011.10.008. URL http://dx.doi.org/10.1016/j.neuron.2011.10.008.

[6] Julien Doyon, Pierre Bellec, Rhonda Amsel, Virginia Penhune, Oury Monchi, Julie Carrier, Stéphane Lehéricy, and Habib Benali. Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behavioural Brain Research*, 199(1):61–75, April 2009. ISSN 0166-4328. doi: 10.1016/j.bbr.2008.11.012. URL http://dx.doi.org/10.1016/j.bbr.2008.11.012.

[7] Risa Kawai, Timothy Markman, Rajesh Poddar, Raymond Ko, Antoniu L. Fantana, Ashesh K. Dhawale, Adam R. Kampff, and Bence P. Ã–lveczky. Motor cortex is required for learning but not for executing a motor skill. *Neuron*, 86(3):800–812, may 2015. doi: 10.1016/j.neuron.2015.03.024. URL https://doi.org/10.1016/j.neuron.2015.03.024.

[8] Chris I. De Zeeuw and Michiel M. Ten Brinke. Motor learning and the cerebellum. *Cold Spring Harbor Perspectives in Biology*, 7(9):a021683, September 2015. ISSN 1943-0264. doi: 10.1101/cshperspect.a021683. URL http://dx.doi.org/10.1101/cshperspect.a021683.

[9] Darby M. Losey, Jay A. Hennig, Emily R. Oby, Matthew D. Golub, Patrick T. Sadtler, Kristin M. Quick, Stephen I. Ryu, Elizabeth C. Tyler-Kabara, Aaron P. Batista, Byron M. Yu, and Steven M. Chase. Learning leaves a memory trace in motor cortex. *Current Biology*, 34(7):1519–1531.e4, April 2024. ISSN 0960-9822. doi: 10.1016/j.cub.2024.03.003. URL http://dx.doi.org/10.1016/j.cub.2024.03.003.

[10] David I. Anderson, Keith R. Lohse, Thiago Costa Videira Lopes, and A. Mark Williams. Individual differences in motor skill learning: Past, present and future. *Human Movement Science*, 78:102818, August 2021. ISSN 0167-9457. doi: 10.1016/j.humov.2021.102818. URL http://dx.doi.org/10.1016/j.humov.2021.102818.

[11] Sally Kelliny, Liying Lin, Isaac Deng, Jing Xiong, Fiona Zhou, Mohammed Al-Hawwas, Larisa Bobrovskaya, and Xin-Fu Zhou. A new approach to model sporadic alzheimer's disease by intracerebroventricular streptozotocin injection in app/ps1 mice. *Molecular Neurobiology*, 58(8):3692–3711, April 2021. ISSN 1559-1182. doi: 10.1007/s12035-021-02338-5. URL http://dx.doi.org/10.1007/s12035-021-02338-5.

[12] Samer Siwani, Arthur S.C. França, Sanja Mikulovic, Amilcar Reis, Markus M. Hilscher, Steven J. Edwards, Richardson N. Leão, Adriano B.L. Tort, and Klas Kullander. OLMα2 cells bidirectionally modulate learning. *Neuron*, 99(2):404–412.e3, July 2018. ISSN 0896-6273. doi: 10.1016/j.neuron.2018.06.022. URL http://dx.doi.org/10.1016/j.neuron.2018.06.022.

[13] Robert Lalonde. The neurobiological basis of spontaneous alternation. *Neuroscience & Biobehavioral Reviews*, 26(1): 91–104, 2002.

[14] Ian N Krout, Alexandria White, and Tim Sampson. Pole test assessment v1. January 2024. doi: 10.17504/protocols.io.5qpvo34k9v4o/v1. URL http://dx.doi.org/10.17504/protocols.io.5qpvo34k9v4o/v1.

[15] Chia-Chien Chen, Anthony Gilmore, and Yi Zuo. Study motor skill learning by single-pellet reaching tasks in mice. *Journal of Visualized Experiments*, (85), March 2014. ISSN 1940-087X. doi: 10.3791/51238. URL http://dx.doi.org/10.3791/51238.

[16] Pauli Virtanen, Ralf Gommers, Travis E. Oliphant, Matt Haberland, Tyler Reddy, David Cournapeau, Evgeni Burovski, Pearu Peterson, Warren Weckesser, Jonathan Bright, Stéfan J. van der Walt, Matthew Brett, Joshua Wilson, K. Jarrod Millman, Nikolay Mayorov, Andrew R. J. Nelson, Eric Jones, Robert Kern, Eric Larson, C J Carey, Ilhan Polat, Yu Feng, Eric W. Moore, Jake VanderPlas, Denis Laxalde, Josef Perktold, Robert Cimrman, Ian Henriksen, E. A. Quintero, Charles R. Harris, Anne M. Archibald, Antônio H. Ribeiro, Fabian Pedregosa, Paul van Mulbregt, and SciPy 1.0 Contributors. SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python. *Nature Methods*, 17:261–272, 2020. doi: 10.1038/s41592-019-0686-2.

[17] Charles R. Harris, K. Jarrod Millman, Stéfan J van der Walt, Ralf Gommers, Pauli Virtanen, David Cournapeau, Eric Wieser, Julian Taylor, Sebastian Berg, Nathaniel J. Smith, Robert Kern, Matti Picus, Stephan Hoyer, Marten H. van Kerkwijk, Matthew Brett, Allan Haldane, Jaime Fernández del Río, Mark Wiebe, Pearu Peterson, Pierre Gérard-Marchant, Kevin Sheppard, Tyler Reddy, Warren Weckesser, Hameer Abbasi, Christoph Gohlke, and Travis E. Oliphant. Array programming with NumPy. *Nature*, 585:357–362, 2020. doi: 10.1038/s41586-020-2649-2.

[18] Thawann Malfatti and Barbara Ciralli. sciscripts, November 2023. URL https://doi.org/10.5281/zenodo.4045872.

[19] John D. Hunter. Matplotlib: A 2d Graphics Environment. *Computing in Science & Engineering*, 9(3):90–95, 2007. ISSN 1521-9615. doi: 10.1109/MCSE.2007.55. URL http://ieeexplore.ieee.org/document/4160265/.

[20] Inkscape Project. Inkscape, 2022. URL https://inkscape.org. Software.

[21] Thawann Malfatti and Barbara Ciralli. Prtbehpred, October 2025. URL https://doi.org/10.5281/zenodo.17484469.

[22] Marianne Leger, Anne Quiedeville, Valentine Bouet, Benoît Haelewyn, Michel Boulouard, Pascale Schumann-Bard, and Thomas Freret. Object recognition test in mice. *Nature Protocols*, 8(12):2531–2537, November 2013. ISSN 1750-2799. doi: 10.1038/nprot.2013.155. URL http://dx.doi.org/10.1038/nprot.2013.155.

[23] Rachael D. Seidler. Differential transfer processes in incremental visuomotor adaptation. *Motor Control*, 9(1):40–58, 1 2005. doi: 10.1123/mcj.9.1.40. URL https://doi.org/10.1123/mcj.9.1.40.

[24] Munekazu Komada, Keizo Takao, and Tsuyoshi Miyakawa. Elevated plus maze for mice. *Journal of Visualized Experiments*, (22), December 2008. ISSN 1940-087X. doi: 10.3791/1088. URL http://dx.doi.org/10.3791/1088.

[25] Fu-Miao Tan, Wei-Peng Teo, Jessie Siew-Pin Leuk, and Alicia M. Goodwill. Effect of habitual physical activity on motor performance and prefrontal cortex activity during implicit motor learning. *PeerJ*, 12:e18217, November 2024. ISSN 2167-8359. doi: 10.7717/peerj.18217. URL http://dx.doi.org/10.7717/peerj.18217.

[26] Arne Nieuwenhuys and Raôul RD Oudejans. Anxiety and performance: perceptual-motor behavior in high-pressure contexts. *Current Opinion in Psychology*, 16:28–33, August 2017. ISSN 2352-250X. doi: 10.1016/j.copsyc.2017.03.019. URL http://dx.doi.org/10.1016/j.copsyc.2017.03.019.