

## DEVELOPMENT OF PATH STEREOTYPY IN A SINGLE DAY IN RATS ON A MULTIPLE-T MAZE

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### INTRODUCTION

Differences between well-learned and newly-learned sequential tasks have been well-established in both non-human primates and in humans (4, 12, 19). Generally, well-learned sequences become more automatic, being performed faster and requiring less attention than novel sequences, and including anticipatory movements not present during novel sequences.

In non-human primates, these differences are generally measured between sequences with which animals have months or years of experience and truly novel sequences which animals have never experienced. For example, Hikosaka et al. (4) describe a visuo-motor hand sequence in which even though animals were proficient at both newly-learned and over-learned sequences (that is, they were not making errors under either condition), animals performing novel and familiar sequences showed a number of significant differences. Novel sequences transferred from the trained to untrained hand relatively easily, while familiar sequences were performed much better by the practiced hand (15, 16). Reversing novel sequences did not slow performance down, while reversing familiar sequences did (15, 16). And finally, animals showed anticipatory movements when performing familiar but not novel sequences (9). This suggests that although animals were performing newly-learned sequences and extensively-learned sequences at near-perfect performance, they were using different strategies for each sequence type.

It has also been demonstrated that rats can show changes in performance strategy, but not within the context of sequence-learning. In rodent navigation studies, rats initially solve tasks using a place-learning or map navigation strategy, and subsequently learn to use a response-based navigation strategy. This has been extensively studied on the plus-maze (14, 17). For example, if rats have been trained for eight days to turn left from the south arm to the west arm, then they turn right when placed for the first time on the north arm (thus going to the same place, but making a different response). In contrast if they have been trained for sixteen days (again from the south arm to the west arm), then they turn left when first placed on the north arm (thus going to a different place, but making the same response).

In humans, changes in performance strategy have been studied within the context of a single day of training using the serial reaction time task (SRTT) (12, 19). In the

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SRTT, subjects are typically given four buttons and an indicator signal (for example, subjects may be instructed to push each button as it lights up). The buttons may be lit up in random order or in a sequence not explicitly shown to the subject. Subjects show decreases in reaction time when the buttons are lit in a sequence, whether they show explicit knowledge of the sequence or not (12, 19). Although some researchers take an alternate viewpoint (e.g., Ref. 5) about whether the sequence is explicitly known to the subject or not, the evidence that error-reduction and reaction-time decreases are consequences of separate systems is overwhelming (1-3, 6-8, 13).

One task which can be used to investigate sequence learning in the rat is the multiple-T maze. Rats trained on multiple-T mazes are required to navigate a sequence of turns in order to obtain a reward. Over multiple days of training, rats learn to complete multiple-T mazes accurately remarkably quickly (making few or no errors after 5-15 trials (see Ref. 11 for review). Subsequent changes in performance strategy on the multiple-T maze, after rats have reached near-perfect performance, have not been described. The experiment described here set out to determine: 1) if rats could learn a new multiple-T maze in a single day and 2) if changes in performance strategy after reaching near-perfect performance could be observed that paralleled the SRTT data described above.

## METHODS

### *Animals.*

Five Brown-Norway-Cross rats obtained from the National Institute on Aging were used (aged 10-12 months at time of experiment). Rats were deprived of food during behavioral training, and were maintained above 80% of their free-feed weight. In most cases, rats received all of their food during the experimental task. Three of the rats had prior experience running for food on a spontaneous-alternation task, while two of the rats did not.

### *The task.*

Rats were trained to run an elevated multiple-T task, the final version of which consisted of 5 Ts arranged sequentially. On either side of the sequence, return rails led from the end of the maze back to the beginning, so that rats ran the maze as a continuous loop. On each return path, two automatic pellet dispensers (Med-Associates, St. Albans VT) were placed, separated by several feet. See Figure 1. On completion of each trial the rat received two 45 mg pellets (Research Diets, New Brunswick NJ) at each dispenser, for a total of 4 pellets per trial. If the rat made an incorrect turn on the final T and ran back along the wrong return rail (thus passing the inactive pair of pellet dispensers), no pellets were delivered, and the rat had to repeat the navigation sequence in order to finish the trial and receive food. Throughout the task, rats were blocked from moving backwards on the maze, but were allowed to make incorrect choices. In practice, rats tended not to turn around and were very rarely blocked.

The maze was constructed of plywood boards measuring 10 cm wide and covered with carpet. Each T consisted of a stem which was 30 cm long, and two choice arms (each 18.5 cm long) oriented at 90 degrees to the stem. The return rails were 212.5 cm long, and were separated by two rails (142.5 cm long), located at either end of the return rails, which led from the navigation sequence to the return rails.

Each trial was defined as starting from the time the animal reached the second feeder (at the end of the return rail). However, rats were not removed from the maze between trials. Thus rats ran the loop continuously. Trials were defined for analysis purposes only.

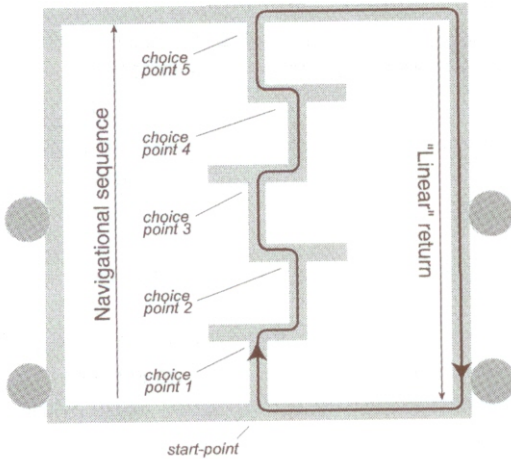


Fig. 1. - *The multiple-T maze.*

General maze layout. The path of the animal is indicated by the dark line. The four filled circles indicate the locations of the feeder-sites. The animal runs a continuous one-way loop, alternating between two reward sites. Each day, the navigational sequence will change. On each day, only one pair of feeders will be active (either in the left or the right side), providing a fifth choice to the navigational sequence.

Initial training was conducted using a shortened maze with only 3 choices. The maze configuration used for each rat was changed daily. After one week, all rats were able to run for food on the maze, and were moved to the 5-T version of the maze. As in 3-T training, each rat was given a new maze configuration each day. In addition, no two rats ran the same maze within a day. Rats were then given 19 daily sessions of 5-T training, in which 15 of the 32 possible maze combinations were presented. Data analysis was restricted to the first 15 days of training, where a new maze was presented each day. Sessions lasted for 40 minutes, in which rats were normally able to run enough trials to maintain their weight.

#### *Data collection.*

Position of the rats on the multiple-T maze was determined using a battery-operated LED backpack constructed in the lab. The LED was secured in an elastic wrap and was fastened together with velcro, which allowed for snug fitting to the rat. Wearing the backpacks, rats were able to move without obvious difficulty, and the LEDs appeared to maintain stable positions on the rat over the course of a session.

LED position was monitored by video tracking input to a Cheetah recording system (Neuralynx, Tucson AZ), sampled at 60 Hz and timestamped with microsecond resolution. Food delivery was controlled with TTL signals generated by in-house software delivered through the computer's serial port. The software monitored the rat's position online and delivered food as the rat reached the appropriate pellet dispenser. Delivery of food pellets was also recorded and timestamped by the Cheetah recording system.

#### *Data analysis.*

From the behavioral data, three measures were examined across sessions: trial duration, errors in maze arm choice, and path stereotypy. Trial duration was defined as the time between successive food deliveries at a pellet dispenser.

Errors were defined as deviations from the median path at each of the choice arms. These deviations occur when rats entered the incorrect choice arm of the T. The median path was defined by using the LED position data from one tenth of the trials from a session, selected at random. The median x position was calculated for every y pixel value (along the length of the navigational sequence) for video tracking data collected in a window which included the navigational sequence but not the return rails. A human determined whether this path was stereotyped (i.e. smooth at each choice point of each T). If the path was not stereotyped, a new set of trials was selected, and a new median path determined. For each trial, deviations from this median path at the choice points were recorded as errors.

Path stereotypy was calculated by examining the correlation of the path across blocks of three trials. To compute path correlation, a path-matrix was defined for each block of three trials. For each path-matrix, each pixel in the video data (480 x 640) was represented with an element set to one if the rat had been at that location, and zero otherwise. This path-matrix was filtered with a 5 x 5 Hamming matrix. A path-correlation-matrix for each session was calculated from the set of path-matrices for a session; strong correlations indicate high similarity in the rat's path between those blocks of trials.

## RESULTS

Rats received 19 sessions of 5-T training in which the maze order was changed daily. The data described below were taken from those sessions (first 15 sessions for each rat, 77 sessions total) in which a new maze was presented each day. In these sessions, the average number of trials completed per day was  $69.4 \pm 11.6$  (mean  $\pm$  SE).

Trial duration was long in the first trials of a day (average of  $141.0 \pm 102.3$  seconds on the first trial), but fell off exponentially to reach a relatively constant value of approximately  $28.9 \pm 2.2$  seconds within 7 trials. The average trial duration remained relatively constant through the remainder of the session.

Mean number of errors made per trial showed a similar pattern. Errors were at chance levels on the first trial ( $2.35 \pm 0.23$  errors; chance = 2.5 errors), and fell off exponentially over the next few trials to reach a steady level for the remainder of the session ( $0.033 \pm 0.024$  within 10-15 trials). These data indicate that the rats were able to learn the maze quickly and efficiently.

While animals reached asymptote in both trial duration, and in number of errors made within 10 trials, they continued to refine their paths over the subsequent 40+ trials. This can be clearly seen in the average path-correlation-matrix (Fig. 2). For early blocks, the path shows little correlation to blocks occurring late in the session. However, after approximately 40 trials, the path correlation becomes more stable, in that the path traveled on the 40th trial and those that follow it are similar to the path traveled through the remainder of the session. Figure 2 shows the mean path correlation using the first 15 sessions of training. Subsequent analyses excluding trials in which errors were made or in which animals were removed from the track did not affect the pattern shown in Figure 2 (data not shown).

## DISCUSSION

Rats were able to learn this task easily; even with minimal pretraining (one week on a 3-T version of the task), animals consistently averaged more than 60 trials per session on a completely novel sequence. Animals also learned to run the route correctly very quickly (making fewer than 2 errors per 10 trials for all trials after trial 8). However, even while failing to make errors, animals continued to refine their paths over the subsequent 40+ trials, showing an increased path-stereotypy with trials.

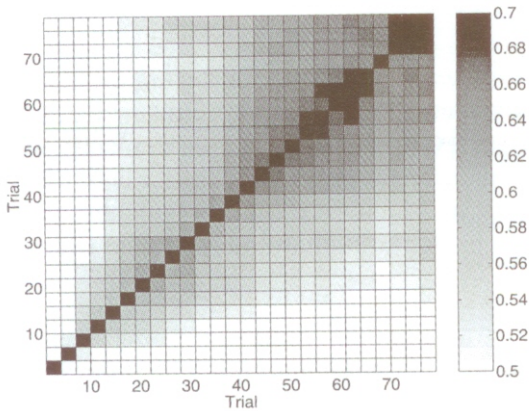


Fig. 2. *Path correlation matrix.*

Shown is the mean correlation matrix for first 15 sessions, 81 trials shown. Each block represents the correlation between blocks of three trials. High correlations indicate similarity in path between blocks. Over the first 20 to 30 laps, blocks show poor correlation to blocks occurring later in the session. After approximately 40 laps, blocks show high and regular correlation to blocks later in the session. These data show that rats adopt a more regular path in this task over the course of 40 laps.

The fast decrease in errors made is consistent with but is slightly faster than found in studies during the first half of the 20th century on rats in elevated T, Y, and U mazes with available distal visual cues (11). The rats in this task were trained on the general task of running a continuous loop for food, and each day had only to learn the specific instantiation of the maze for the day. Rats who have been pre-trained on tasks, thus requiring only parameter instantiation for error-free trials, can show one-trial learning. For example, in the hidden-platform water maze, rats are trained to find a hidden platform within an opaque pool of water (10). If the location of the platform is changed from day to day, but remains constant within a day, rats show one-trial learning, finding the platform accurately on the second trial of the day (18).

Humans generally show a reduction in reaction-time on repeated sequence tasks (12). A corresponding reduction in trial-duration was not observed in the present study in rats running a multiple-T maze. Animals did, however, show a decrease in path-length, and in running speed (data not shown). The lack of a reduction in trial-duration is likely to be a consequence of the decrease in running speed overwhelming the decrease in path length.

The development of path-stereotypy is particularly interesting because it has been shown that patients with striatal damage do not show sequence learning in the serial reaction-time task (SRTT) (Parkinson's patients: (2, 3, 6), Huntington's patients: (7), see Ref. 1 for review). In contrast, patients with diseases typically involving declarative memory systems are able to learn the SRTT (Alzheimer's patients: (3, 8), Korsakoff's patients: (19), see Ref. 1 for review). Similarly, anticholinergic drugs (such as scopolamine) which are known to affect the ability to recall stimuli do not affect the SRTT (13).

Whether the decrease in errors-made (dropping to asymptote within 10 trials) and the increase in stereotypy (reaching asymptote only after 40 trials) are indicative of two separate learning systems or an aspect of a single learning system still remains to be shown. It is hoped that this task may enable the study of a change in performance strategy within a single day, thereby simplifying the elucidation of the mechanisms underlying this phenomenon.

## SUMMARY

Humans and animals trained on sequential reaction tasks show decreases in reaction time and increases in anticipatory movements even long after they have ceased to make errors. Humans show these changes even when they do not explicitly recognize that they performed a repeating sequence. We have developed a task which rats learn to perform error-free quickly, but in which they continue to show path-refinement on a single day. This task may enable the study of performance strategy changes occurring within a single day.

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