Behavioral shaping of rhesus macaques using the Cambridge neuropsychological automated testing battery


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ABSTRACT

Background: The Cambridge neuropsychological test automated battery (CANTAB) is a set of computerized visuospatial tests used to probe cognition in humans. The non-human primate (NHP) version of the battery is a valuable translational research tool to quantify cognitive changes in NHP models of disease by allowing direct comparison with performance data from human patient populations. One limitation is the long training times required for NHPs to reach appropriate levels of task performance, which is prohibitive for high throughput experimental designs.

New method: We report a new training regimen to teach NHPs a subset of CANTAB cognitive tasks using a method of successive approximations (shaping), where rewarded behaviors progressively approximate the goal behavior, and sequential task learning is used to build upon previously learned rules. Using this refined method, we taught 9 adult rhesus macaques to perform three tasks: the self-ordered spatial search (SOSS), delayed match-to-sample (DMTS), and paired associative learning (PAL) tasks.

Results and comparison with existing methods: NHPs learned all three cognitive tasks in approximately 130 training sessions, roughly 200 sessions faster than previously published training times. NHPs were able to perform each task to a stable level of performance (> 80 % correct) enabling their use in future cognitive experiments.

Conclusions: Our approach of behavioral shaping reduced the time to train NHPs to performance criteria on SOSS, DMTS, and PAL tasks. This allows efficient use of the NHP-adapted CANTAB to compare cognitive changes in NHP models of neurological disease with those observed in human patient populations.

1. Introduction

The Cambridge neuropsychological test automated battery (CANTAB) is a set of language free, visuospatial tasks of learning, memory, and attention developed to test cognition (Robbins et al., 1994; Sahakian and Owen, 1992). The goal of the CANTAB is to aid in the screening and early detection of neurological conditions, with a particular emphasis on neurodegenerative diseases. The CANTAB has been validated through numerous reports of cognitive performance data from various human patient populations, including those with Alzheimer’s disease (Swainson et al., 2001; Junkkila et al., 2012; Égerházi et al., 2007) (AD), mild cognitive impairment (Swainson et al., 2001; Blackwell et al., 2004) (MCI), Huntington’s disease (Lawrence et al., 1998; Lawrence et al., 1996; Lange et al., 1995) (HD), and Parkinson’s disease (Owen et al., 1993; Morris et al., 1988; Lewis et al., 2003) (PD). The power of the CANTAB device lies in the large normative database available that provides benchmark performance across age on all tasks. Due to the language free design aspect of these tasks, the CANTAB is also used to study cognition in non-human primates (NHPs). Marmosets (Spinelli et al., 2005; Spinelli et al., 2004; Nakako et al., 2013a), cynomolgus (Kromrey et al., 2015a; Kromrey et al., 2015b), and rhesus macaques (Taffe et al., 1999; Weed et al., 2004; Wright et al., 2013; Weed et al., 1999; Marino and Levy, 2019) have all been successfully trained to perform various NHP-adapted versions of the CANTAB tasks, and the CANTAB has also been used to quantify cognitive changes in NHP models of disease (Taffe et al., 1999; Weed et al., 2004; Schneider et al., 2015; Schneider, 2006; Nagahara et al., 2010). The ability to test the same cognitive tasks in both NHP disease models and human patient populations makes the CANTAB a valuable translational research tool.

The CANTAB itself is comprised of several independent neuropsychological tests that target different aspects of attention, cognitive flexibility, learning, and memory performance. In this study we focused on the self-ordered spatial search (SOSS), delayed match-to-sample...
Unfortunately, despite the useful applications of the CANTAB, the amount of time and effort required to train NHPs to perform the tasks at a level of performance that allows for measurement of any cognitive impairments is quite significant, often taking many months. While humans do not require any prior training to perform the CANTAB tasks, it can take up to several months of training for NHPs to be able to perform a single CANTAB task (Spinelli et al., 2004; Kromrey et al., 2015b; Weed et al., 1999), and similarly long training times can be required for DMTS and PAL tasks (Spinelli et al., 2005; Spinelli et al., 2004; Kromrey et al., 2015a; Kromrey et al., 2015b; Nagahara et al., 2010; Nakako et al., 2013b), meaning it could take well over a year to train a given animal on all 3 tasks. Training time for NHPs is the major barrier to utilizing the CANTAB as a potential translational research tool, as it is neither economical nor practical to have to train new cohorts of animals for many months for each new experiment. In order for the CANTAB to be effectively used in high throughput experiments, the training times for animals must be reduced. Therefore, our aim is to develop a training protocol for NHPs that would allow them to learn translationally relevant CANTAB tasks in the shortest possible time to optimize resources.

With a goal of training NHPs faster, we developed a training protocol that utilized behavioral shaping and sequential learning. Behavioral shaping is the concept of establishing a wanted task behavior by first presenting the subject with a different, typically simpler, task and then continuously reinforcing the behavior while simultaneously altering the task so that it more closely resembles the final desired task (Peterson, 2004; Skinner, 1953). This method of successive approximations can be used to train NHPs gradually until they perform the actual task using the standard CANTAB stimulus sets. Behavioral shaping has often been used to expedite training times in other behavioral tasks, such as saccadic eye movement tasks (Bell et al., 2000), but to our knowledge has not yet been reported as a method to facilitate CANTAB training. In addition to using shaping, our method also teaches the CANTAB tasks sequentially, such that learning in future tasks are builds upon previously learned rules from earlier tasks. The sequential teaching of tasks differs from previously published CANTAB studies, which traditionally taught all CANTAB tasks concurrently (Weed et al., 1999; Taffe et al., 2002a). We hypothesize that by using behavioral shaping and sequential task learning we can hasten CANTAB task learning. Here we present a methodology of training NHPs on the SOSS, DMTS, and PAL CANTAB tasks to stable high performance (> 80 % correct) in a faster time frame than previously reported. Additionally, we examined whether liquid or food rewards were more effective as trial motivators for NHPs, and found that both produced similar training times.

2. Materials and methods

2.1. Subjects

All animal care and experimental procedures were approved by the Queen’s University Animal Care Committee in accordance with the

<table>
<thead>
<tr>
<th>Animal Symbol</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Reward Type</th>
<th>Previous Conditions</th>
<th>Touch Training</th>
<th>2-Box 3-Box 4-Box</th>
<th>1-Stim 2-Stim 3-Stim</th>
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<tr>
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<td>-</td>
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<td>Naive</td>
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<td>Naive</td>
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<td>2</td>
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<td>Naive</td>
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<td>22</td>
<td>2</td>
</tr>
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<td>Naive</td>
<td>54 28 13</td>
<td>27</td>
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<td>Naive</td>
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<td>14</td>
<td>1</td>
</tr>
<tr>
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<td>Naive</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>Water</td>
<td>Naive</td>
<td>11 10 13</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1 Summary table of the demographics of each NHP trained on the CANTAB tasks as well as their respective average training times on the individual tasks.
guidelines of the Canadian Council on Animal Care. Nine adult rhesus monkeys (*Macaca mulatta*), between 8 and 15 years of age at the onset of training (8 male and 1 female), were used in this study (Table 1). All NHPs were naïve to the CANTAB at the start of the study. Most were also experimentally naïve with the exception of NHPs 1, 3, and 4 who had previously been used in saccadic tasks for liquid reward involving neurophysiological recordings from the superior colliculus, frontal eye fields, and primary visual cortex (White et al., 2017a; White et al., 2017b; Wang et al., 2012; Wang et al., 2014). These previous eye movement tasks had no commonalities to the CANTAB tasks presented here with the obvious exception of both being reward-based tasks requiring a behavioral response to stimuli on a computer screen, but this only affected the initial time to learn reward acceptance and touch training, not task-specific training times. Because all of the NHPs used in this study were destined for different experiments, the specific CANTAB tasks a given NHP learned depended on what was required for their respective future experiment. As such, only a subset of animals were trained on all three tasks. Nine animals were trained on the SOSS task, 5 on the DMTS, and 4 on the PAL task (Table 1). In the PAL task, all 4 were trained to criteria on the 2-stimuli variation, and 2 of these 4 were also trained to criteria on the 3-stimuli variation.

2.2. General CANTAB parameters

CANTAB sessions were run at the cage-side with the NHPs in their home cages. All animals were single-housed in large pens, but were in a common room with other animals. For each session, a specialized cart containing the CANTAB apparatus (Model 80950A; Lafayette Instrument Company, Lafayette, IN), computer screen, keyboard, and mouse was attached to the front of the NHP’s pen (Fig. 1A–B). Real time experimental control was achieved through the WHISKER server software (Lafayette Instruments) which displayed the visual stimuli, recorded behavioural responses, and controlled reward delivery. The dimensions of the cart were 104.5 cm high × 72.5 cm wide × 53 cm deep so as to have the CANTAB’s touch-screen align to a removable plexiglass window that is part of the NHP cage (56 cm × 42.5 cm). When ready to initiate trials, the plexiglass window was removed and replaced with a stainless steel grid with 2.5 × 2.5 cm openings to allow the NHP to reach through and touch the screen but prevent them from being able to swipe or place multiple fingers at once on the screen (Fig. 1C). This refinement was important as it limited NHPs to touching the CANTAB screen with a single finger, which greatly reduced the occurrence of erroneous touches registered by the CANTAB caused by NHPs swiping the screen with their whole paw. While this refinement was present throughout the individual task training of the NHPs, it would only be expected to facilitate touch training acquisition and not task-specific rule sets. To allow for the possibility of both food and liquid reward, the base CANTAB model was modified by adding the Rhesus lick tube (Model 80662B; Lafayette Instrument Company) and peristaltic pump for liquid reward (Model 80204 P; Lafayette Instrument Company).

NHPs were kept on a strict 12 h light-dark cycle (7:00 am lights on) and CANTAB testing was conducted between 8:30 am and 6:30 pm Monday to Friday, with sessions typically lasting 2 h in duration (NHPs averaged 182.4 ± 22.70 trials a session). Animals were limited to one session per day and typically ran at the same time every day. Each animal was tested 5 days a week. The experimenter was only present to facilitate training during the initial touch training phase. To motivate NHPs to attempt trials, NHPs were either placed on a water-restricted (n = 6) or food-delayed (n = 3) regime (Table 1). Water restricted NHPs received no water each morning on CANTAB session days and received 1.5 ml of diluted Gatorade (1:5 dilution of G2 Fruit Punch Gatorade with water) as a reward for each correct trial. These animals were rehydrated with ad libidum access to water on Friday afternoon and Saturday before being restricted on Sunday again in preparation for the weekly training. For food delayed training, afternoon chow and fresh fruits were delayed until after the CANTAB session had concluded, and these NHPs were rewarded with one 0.190 g fruit pellet (Bio-Serv, Flemington, NJ; Cat # F05798) on each correct trial. Both the water and food rewards were paired with a tone (1000 Hz for 1 s; 85 dB) to designate a correct trial whereas incorrect trials were paired with a lower frequency tone (40 Hz for 2 s; 85 dB) and followed by a 10 s ‘time-out’ black screen before the next trial was initiated. Trials were initiated spontaneously and in between all trials there was a 2–3 second interval interval. Each individual trial had a 10-minute time limit before being scored as incomplete and moving on to the next trial. Incomplete responses were not included in performance calculations. Training performance criteria cut-offs were determined based upon being approximately halfway between random chance and perfect performance (60–80 % correct on a single session), so as to establish baseline task performance that would be sensitive to future neurological manipulation experiments. As such, more difficult tasks with lower random chance levels tended to have lower performance criteria cut-offs as this hastened training while still generating a baseline task performance that would be able to detect any potential neurological deficit. Once NHPs reached criteria on a given task, they were moved to the next sequential task and no longer performed the previous tasks until after all tasks had been learned.

Once NHPs had reached criteria performance level on all tasks, CANTAB performance was maintained prior to experimental with CANTAB sessions consisting of all trained tasks in sequential blocks. Every session started with 20 trials of a sensorimotor control task, then 15 trials each of the 2-, 3-, and 4-box SOSS task (total of 45 trials), then 30 DMTS trials (5 trials at each delay using ‘hard’ stimuli set), and finally 40 trials of the PAL task (10 trials of 1 stimuli, 10 trials of 2 stimuli-2 distractor, 10 trials of 2 stimuli-3 distractor, and 10 trials of 3 stimuli).

2.3. CANTAB touch training

NHPs were first introduced to the CANTAB apparatus and familiarized with accepting rewards by utilizing the built-in reinforcement familiarization paradigm to freely reward the NHP at random time intervals (240 rewards over two hours, variable-time 30-second reward paradigm) (Weed et al., 1999). Once NHPs were acclimated to the CANTAB apparatus, touches to the screen were ‘baited’ with either apple or pear pulp. During this stage, the CANTAB screen was completely filled with a purple square that recognized all touches as correct and rewarded them appropriately. After the NHP learned that touching the screen was rewarded, the size of the purple square was gradually decreased until the NHPs accurately touched a 3 × 3 cm square appearing at random screen locations rather than just at the center (Supplemental Fig. 1). The stimulus was reduced in size (approximately 20 % reduction) each time the NHP produced 10 correct trials in a row until the final size (3 × 3 cm). Touches made to the screen but not to the stimulus location did not result in an incorrect trial and the trial continued until the NHP touched the stimulus. NHPs were deemed to be touch trained when they managed to complete over 100 trials at the final stimulus size.

2.4. Self-ordered spatial search (SOSS) task

In the SOSS task (Fig. 2), the NHP was presented with a variable number of 3 × 3 cm blue box stimuli (2, 3, or 4 boxes) at different locations on the screen and the NHP was required to touch to each box once and only once (Fig. 2A). When a stimulus was touched, the touched box briefly flashed white (0.1 s) before all stimuli disappeared for a variable amount of time (1, 2, or 3 s). The boxes then reappeared in the same color and location as prior to the touch (Fig. 2B). If the NHP touched the same box twice within a trial, the trial was terminated and scored as incorrect. If each box was touched once and only once, the trial was scored as correct and the NHP was appropriately rewarded at the conclusion of the trial.
To train the NHPs on the SOSS task, a shaping strategy was used. To start, the NHPs were given the 2-box condition of the task but with the variation that when a box was successfully selected, it ‘permanently disappeared’ for the duration of the trial and only the other untouched stimulus reappeared (Fig. 2C). This made the trial impossible to get incorrect (error-less learning) and forced the NHP to always touch a previously untouched stimulus. For the next step, the touched object did not disappear but instead changed color to white (Fig. 2D) to indicate it had been previously touched, providing an overt memory cue for previously touched items. Once an NHP achieved over 80 % correct on the ‘white-replaced’ trials, another variation of the task was introduced, this time with the previously touched stimulus being replaced with a ‘light blue’ box (termed LightBlue Box; Fig. 2E; RGB [100,100,255]). Once performance on this task surpassed 80 % correct, a variation with a ‘darker blue’ box was added (termed DarkBlue Box; Fig. 2F; RGB [50,50,255]), and once the NHP achieved 80 % correct on this variation, the ‘unaltered’ task was introduced (RGB [0,0,255]). As task variations were added they were interleaved in small trial blocks with the pre-existing task variations in the order of ‘permanently disappear’, ‘white-replaced’, ‘light blue-replaced’, ‘darker blue-replaced’, then ‘unaltered’. Sessions consisted of 10 trial blocks of these task variations in this order repeating for as many trials as the NHP completed. The time delay between choice phases of the task was kept at 1 s during the training variations, but once 80 % accuracy was achieved on the unaltered task, 2 and 3 s conditions were introduced. Both the 3- and 4-box conditions of the task were taught sequentially in the same approach after the NHP finished with the 2-box condition. The only difference was that only 60 % accuracy was required to transition from the darker blue-replaced boxes to the unaltered task in the 4-box condition, and for both 3- and 4-box conditions 60 % accuracy was required to incorporate 2- and 3-second delay conditions as opposed to the 80 % used in the 2-box condition.

Fig. 1. A-B: Photographs of the CANTAB apparatus and specialized cart to allow NHPs to work at the cage-side. The cart is attached to the caging use the two red toggle clamps. C: Photograph of the CANTAB apparatus and stainless steel grid from inside the NHP’s pen.
2.5. Delayed match-to-sample (DMTS) task

In the DMTS task (Fig. 3), the NHP was first presented with a single unique stimulus that the NHP was required to touch to proceed (Fig. 3A). Once touched, the screen went blank for a variable amount of time (either 1, 2, 4, 8, or 16 s) to serve as a memory delay, after which the original stimulus as well as a variable number of non-matching distractor stimuli (0, 1 or 3) appeared on the screen. The NHP was required to touch the matching stimulus and avoid the non-matching distractors. If a touch was made to the non-matching distractors, the trial was scored as incorrect. During the choice phase of the trial, the NHP had a maximum of 10 s to select a stimulus before the trial terminated and was scored as incomplete. The stimuli used in this task consisted of two sets: one with easily distinguishable shapes that was used for training and for an ‘easy’ version of the task (Fig. 3A; CANTAB built-in stimulus set: pal0), and another with abstract patterns consisting of four rectangular quadrants of different colors and shapes, used for a ‘hard’ version of the task (Fig. 3E; CANTAB built-in stimulus set: mdms0). For each of these stimuli, any one quadrant could appear as one of seven distinct colors and the quadrants were shuffled resulting in over 70 million unique stimuli.

To begin learning the DMTS task, the NHPs were first started on a variation of the task that used the ‘easy’ stimulus set at a 1 s memory delay and had no distractors in the choice phase of the trial, so the only option was the correct matching stimulus (Fig. 3B). After two sessions on the ‘no distractors’ condition, a variation containing only one distractor (‘single distractor’ condition) was added into the sessions, with blocks of the two variations alternated (Fig. 3C; 20 trials per block). During this step, it was of particular importance to monitor whether the NHP developed a side-bias to their touches. NHPs often began only selecting one side during the choice phase effectively forgoing learning the task for the guaranteed 50 % correct reward rate (while not explicitly defined, intervention to correct the side bias was typically initiated when an NHP was observed touching one side for > 80 % of the trials). If such side-biased behavior was observed, the task was changed the following day so that incorrect trials were continuously repeated until the NHP got it correct. This forced the NHP to touch both possible stimulus locations in order to continue getting rewards. This variation was stopped immediately after the side-bias was broken to avoid the development of a win-stay, lose-switch strategy (variation stopped when NHP had returned to within 40–60 % side choice). This ‘single distractor’ condition was continued until the NHP reached over 80 % accuracy at which point the ‘no distractors’ condition was removed and additional trials of the ‘single distractor’ condition with delays of 2 and 4 s were added in addition to the 1 s delay trials. Once the NHP achieved over 80 % correct on the new delay trials, 8 and 16 s trials were added (Fig. 3D). Once at least 60 % accuracy had been achieved on all delays, the number of distractors was increased to 3 and the ‘hard’ stimulus set was added (Fig. 3E). The final DMTS behavior was tested using 3 distractors using the ‘hard stimuli set.'
2.6. Paired associates learning (PAL) task

In the PAL task (Fig. 4), the NHP was first presented with a series of individual stimuli in specific locations that the NHP was required to touch in order to proceed. The number of stimuli ranged from 1 to 3 depending on task difficulty. After the NHP made all of the initial sample phase touches, the same stimuli were re-presented in a random order but in the presence of identical distractor objects in other locations in addition to the original observed location. To get the trial correct, the NHP was required to touch only the stimulus in the original position, not the identical distractors in other locations. The NHP had to successfully repeat this for each of the initially presented stimuli to get the trial correct, any touches to the incorrectly positioned distractors resulted in the trial being scored as incorrect. The NHP was rewarded for each choice phase correctly performed, not just at the end of the entire trial. The stimuli used in the PAL task were the same as the stimuli used in the 'easy' variation of the DMTS task described previously (Fig. 3A). Unlike other tasks, if an NHP got a trial of the PAL task
incorrect, it was repeated until either the NHP got the entire trial correct or a maximum of 6 attempts were incorrectly completed. Between each sample and choice phase there was a 1 s delay.

The training paradigms for the PAL task was very similar to that originally published by Taffe et al. (2004) but with slight modifications. To summarize, NHPs were taught this task by starting on a one stimulus condition during the sample phase and during the choice phase there were no distractors (1stim-0dist condition), during this variation it was not possible for the NHP to get a trial incorrect (Fig. 5A). Once familiarized with the task, the NHP was switched to a variation with a single distractor (1stim-1dist condition; Fig. 5B) and training continued until 80 % correct trial performance on the first attempt was achieved. The NHP was then started on a two distractor variant (1stim-2dist condition) until 80 % accuracy was again achieved (Fig. 5C). The two sample stimuli condition of the PAL task was then introduced with no distractors (2stim-0dist condition) and this was typically the rate limiting step in training NHPs on this task (Fig. 5D). The NHPs were kept on this variation until the average number of attempts required to get a trial correct was < 2. Once achieved, a two sample variation with one distractor (2stim-1dist condition) was added in addition to the zero distractor variation (Fig. 5E). This continued until the NHP achieved an average of 2 attempts required to correctly complete a given trial. Finally, the NHP was started on the most difficult variation of the task, which involved three sample stimuli with no distractors (3stim-0dist condition; Fig. 5F). Once the NHP had learned all the variations of the PAL task, performance was maintained using a battery consisting of 10 trial blocks of 1 stimuli-2 distractor, 2 stimuli-0 distractor, 2 stimuli-1 distractor, and 3 stimuli-0 distractor variations.

### 2.7. Data analysis

Animals were trained on one task at a time until it was mastered, and the training times presented below represent the total number of sessions that each animal required to reach performance criteria (60–80 % correct depending on task) on the final step of each task’s training progression. Composite training figures were generated by averaging the length of training each animal required for a given training step as well as averaging the percent correct of all animals on each respective training session within a given training step. All values are presented as the mean ± standard error. Data normality was tested using a one-sample Kolmogorov-Smirnov test and no distribution data significantly differed from normal (all p > 0.05). Differences between task performance and random chance were established using a one-sample student’s t test, differences between food-delayed and water-restricted NHPs were analyzed using an independent samples t test, and training time differences between task variations were analyzed using a one-way ANOVA.

### 3. Results

#### 3.1. CANTAB training overview

The NHPs were taught the tasks in sequential order starting with initial touch training, followed by the SOSS 2-box condition, the SOSS 3-box condition, the DMTS task, the SOSS 4-box condition, and then finally the 1-, 2-, and 3-stimuli conditions of the PAL task (Fig. 6B). For those NHPs that were only trained on the SOSS task, they moved straight from the SOSS 3-box condition to the SOSS 4-box condition in training, omitting the DMTS task training. Training of the DMTS task occurred between the 3-box and 4-box SOSS task conditions as it was decided post hoc to train NHPs on the 4-box SOSS condition, and thus going forward the order was kept the same to enable between animal comparisons of training times. It took approximately 130 training sessions to master the three tasks, starting from the first touch training session to the final session performing the 3-stimuli PAL condition (Fig. 6B). Our training times were substantially shorter when compared to the currently published average training time of approximately 330 training sessions for these same tasks (Fig. 6A) (Weed et al., 1999; Taffe et al., 2004).

#### 3.2. Initial touch training

All animals were first taught to make touches to randomly appearing 3 × 3 cm targets on the touchscreen apparatus and receive rewards for correct trials. The average number of training sessions required for the animals to learn to touch the targets was 5.6 ± 0.7 sessions, with the fastest NHP acquiring the skill in only 2 training sessions and the slowest requiring 9 sessions (Supplemental Fig. 2). There was no significant difference in touch training times between animals that were water-restricted (6.3 ± 0.7 sessions) and those that were food-delayed (4.0 ± 1.2 sessions; t(7) = 1.81, p = 0.11).

#### 3.3. SOSS task

The composite percent correct 2 box SOSS condition training curves for all NHPs (n = 9) is shown in Fig. 7A. Manipulating the color of the previously touched stimuli so that it turned permanently white (Fig. 2D), resulted in a rapid increase in correct trials with NHPs averaging over 80 % correct after only 5–6 training sessions. Adding interleaving variations of the task using closer approximations of the unaltered stimuli (LightBlue and DarkBlue stimuli, Fig. 2E and F respectively) resulted in no erosion of the percent correct with NHPs maintaining high task accuracy, well above what would be predicted by chance alone (77.7 % correct vs. 50 % chance; t(8) = 6.37, p < 0.001). This higher than chance performance on the first session of a more difficult task variation suggests that the NHP is retaining previously learned rules and generalizing them to the newer task variation. This pattern continued as NHPs were transitioned to the unaltered stimuli task variation as well, managing to obtain > 80 % correct performance on the first training session, once again suggesting that the previous shaping trial variations taught the NHPs a set of rules that were carried forward to new task variants. Additionally, the fact that there was no decrease in task accuracy when introducing the variations using the less distinctive stimuli suggested that the training of NHPs on the 2-box SOSS condition could be even further optimized and training times reduced. Overall, using this shaping teaching method, training of the 2-box SOSS task (unaltered version with > 80 % correct) took an average of 31.0 ± 6.1 training sessions (range: 11–62 sessions; Fig. 7B).
The 3-box SOSS condition training curves (Fig. 7C) also showed that NHPs rapidly increased performance on the white box variation within very few trial sessions and even had performance well above chance starting at the first training session (70.1% correct vs. 22% chance; $t(8) = 8.97$, $p < 0.001$). This high baseline performance was presumably due to starting each animal with a task variation in which the stimuli vanish when touched (Fig. 2C) in combination with the NHPs applying previously learned concepts from the 2-box condition. In contrast to the 2-box condition, performance decreased when moving to a less differentiated stimuli set before steadily climbing in a similar manner to the white box variation, suggesting that each step was noticeably more difficult from the NHP’s perspective. Despite this, NHPs managed to achieve the > 80% correct criteria threshold within only 2−3 training sessions on each of these training steps, and when finally introduced to the unaltered task were able to perform at > 60% correct on the first session. In total, it took NHPs 23.2 ± 3.8 training sessions to fully learn the 3-box condition (range: 10–42 sessions; Fig. 7D).

Fig. 5. A–F: Schematic illustrations of the different task variations used to train an NHP on the PAL task. NHPs first start on the variation where they are only presented with a single choice stimulus and no distractors in the choice phase (A). Once the NHP is familiarized with the task, an additional distractor is added (B), and then another distractor is added to a total of two (C). After sufficient accuracy has been achieved on the one stimulus variations, the NHP is moved to the two choice stimuli variation (D). This presents the rate limiting step for learning. Once learned, an additional distractor is added (E), and then finally the NHP is moved to a three choice stimuli variation (F). Green arrows represent a correct choice, whereas the red arrows represent an incorrect choice.
Fig. 6. Bar graphs showing the current published average number of CANTAB daily sessions required to teach all CANTAB tasks (A) and individual task training times using our methodology (B). Each color represents an individual NHP trained on that given CANTAB task.

Fig. 7. A,C,E: Composite training curves showing the aggregate performance of all trained NHPs on the different task variations used to shape behavior on the 2-box (A), 3-box (C), and 4-box (E) SOSS task conditions. Dashed lines represent performance criteria thresholds for advancing to the next task variation. B,D,F: Plots showing the individual training times in number of sessions required for NHPs to reach final performance criteria in the 2-box (B), 3-box (D), and 4-box (F) SOSS task conditions. The larger solid line represents the mean training time for all animals and the smaller solid lines represent the standard error of the mean. The composite plots only show the initial training using the 1-second delay condition, following this each animal had additional training sessions to learn the 2- and 3-second delay conditions. Additionally, prior to these training curves the animals were started on 2-3 sessions of a task variation where the boxes vanished when touched (always 100 % correct). Together these contribute to why the average training times (B,D, and F) appear to be longer than the aggregate training curves would suggest (A,C, and E).
(Fig. 7E). As with the 3-box condition, the starting performance of the NHPs on the 4-box condition white box variation was much higher than what would be predicted by chance alone (69.6% correct versus 9.4% chance; t(6) = 11.73, p < 0.001). Although NHP performance decreased as they moved towards harder variations of the 4-box condition, at each step they progressively improved and when finally introduced to the unaltered task variation, first session performance was well above chance levels (45.2% correct versus 9.4% chance; t(6) = 3.81, p < 0.009). Again this indicates NHPs were employing learned task rule sets that they had previously acquired during the shaping trial variations. Overall, NHPs were able to learn the 4-box condition in 34.4 ± 8.3 sessions (range: 9–71 sessions; Fig. 7F). Although there were no significant differences, animals under water-restriction tended to learn the task faster than those under food-delay; this was observed for 2-box (26.3 ± 7.6 water-restricted sessions versus 40.3 ± 9.5 food-delayed sessions; t(7) = 1.09, p = 0.31), 3-box (20.0 ± 5.2 sessions versus 29.7 ± 2.7 sessions; t(7) = 1.24, p = 0.25), and 4-box conditions (27.6 ± 9.1 sessions versus 45.3 ± 17.1 sessions; t(6) = 0.98, p = 0.37).

3.4. DMTS task

After two training sessions where the NHPs were given a no distractor version of the DMTS task (always 100% correct), 5 NHPs were started solely on a 1-s, 1-distractor delay version of the task using the easily distinguishable stimuli set. Using these parameters, NHPs quickly reached over 80% correct performance within 10 training sessions (Fig. 8A). Introduction of longer delays (2-, 4-, 8-, and 16-seconds) after establishing the task using the 1-s delay variation resulted in little decrease in performance with the exception of the 16-second delay. Transition to the 3-distractor condition of the DMTS task (all delays) resulted in a predictable drop in performance, but was still well above chance levels (51.4% correct versus 25% chance; t(4) = 3.99, p = 0.016) and improved to 74.3% correct within 5 sessions. This indicated that the NHPs had learned and retained the task relevant rules from previous variations. Overall, from starting with a task-naive NHP, the average time taken to learn the 3-distractor condition of the DMTS task using the ‘hard’ stimuli set took 19.2 ± 2.6 training sessions; the quickest was 11 sessions and the longest was 27 sessions (Fig. 8B). There was no appreciable difference between the training times of those under water-restriction (18.5 ± 0.3 sessions) and those being food-delayed (19.7 ± 4.7 sessions; t(3) = 0.19, p = 0.86).

3.5. PAL task

To begin teaching the PAL task to NHPs (n = 4), they were given 2 training sessions of a PAL task variation with no distractor and only 1 stimulus, making the trial impossible to get incorrect. After these two initial sessions, the NHPs were transitioned to a 1 stimulus-1 distractor variation and although starting with performance near that predicted by chance (51.6% correct vs. 50% chance), by the third training session performance was already > 80% correct (Fig. 9A). Initiation of the 2 stimuli condition represented a large increase in task difficulty as the choice phases appear in an independent order of when the stimuli were presented and thus initial performance was poor and training was slow. Regardless, NHPs showed a steady improvement in 2 stimuli-0 distractor task performance until reaching 74% correct on first attempt over an average of 35 training sessions. This step represented the major rate limiting step in NHPs learning the PAL task and took significantly longer to acquire than either the 1 stimulus or 3 stimuli conditions of the PAL task (45.5 ± 7.8 sessions for the 2 stimuli condition versus 21.3 ± 0.9 sessions for the 1 stimulus condition and 25.5 ± 0.5 sessions for the 3 stimuli conditions; F(2,7) = 6.17, p = 0.028; Fig. 9B–D). As with both the DMTS and SOSS tasks, there were no statistically significant differences between water-restricted and food-delayed NHPs on their acquisition of the PAL task conditions. Acquisition times for the 1 stimulus and 3 stimuli conditions were similar between the two groups (1 stimulus condition: 21.5 ± 0.3 water-restricted sessions versus 20.5 ± 1.2 food-delayed sessions, t(2) = 0.24, p = 0.83; 3 stimuli condition: 26 water-restricted sessions versus 25 food-delayed sessions), whereas the acquisition times for the 2 stimulus condition trended towards food-delayed animals acquiring the task quicker (58 ± 3.5 water-restricted sessions versus 33 ± 3.3 food-delayed sessions, t(2) = 3.47, p = 0.07).

4. Discussion

Here, we have described a comprehensive set of training methodologies that were used to accelerate the training of rhesus macaques on the SOSS, DMTS, and PAL CANTAB tasks. We used a combination of behavioral shaping and sequential task training approaches to rapidly and efficiently train NHPs to stable, high performance levels on multiple conditions in each of the tasks. Throughout the training paradigm (Fig. 6A), when NHPs were moved to more difficult task variations of the same task they consistently showed first session performance greater than what would be predicted by chance alone (Figs. 7–9), indicating that each training step was successfully preparing the NHP for future task variations. This was observed both within a given task as well as across tasks, suggesting that the tasks shared common features and thus using sequential task learning builds upon previously learned concepts and expedites learning.

4.1. Relation to previous studies

Comparing the overall training time to teach all three CANTAB tasks using our methodology against what was published previously suggests that our method significantly reduced the time required. There is not a great deal of literature describing NHP CANTAB training times in-depth, but the studies we found suggested that training a CANTAB-

![Fig. 8](image-url) A: Composite training curve showing the aggregate performance of all trained NHPs on the different task variations of the DMTS task. Prior to starting the 1-second 1-distractor variation, each animal is started on two sessions of no distractor DMTS, which will always have 100% accuracy. The starting training sessions are below what would be expected by chance alone due to the presence of side bias in the animals that must be overcome before learning the task. The 3 distractor values represent a composite of all 1 distractor delays. B: The individual training times in number of sessions required for each NHP to reach final performance criteria in the DMTS 3-distractor condition using the ‘hard’ stimuli set. The larger solid line represents the mean training time for all animals and the smaller solid lines represent the standard error of the mean. Dashed lines represent performance criteria thresholds for advancing to the next task variation.
naïve NHP to performance criteria on the SOSS, DMTS, and PAL tasks would require approximately 330 training sessions (Weed et al., 1999; Taffe et al., 2004). In contrast, it took an average of 130 training sessions for our NHPs to learn these same tasks (Fig. 6B). We were able to train NHPs on 2-, 3-, and 4-box conditions of the SOSS task all at 1-, 2-, and 3-second delays in an average of 88.6 training sessions, much faster than the previously published average training time of NHPs on the 4-box condition at a fixed 2-second delay of 134.1 training sessions (Weed et al., 1999). Our faster training times were presumably due to the fact that we used a training paradigm where NHPs were taught the task by using progressive task variations that more closely resembled the actual task rather than just exposing the NHPs to the unaltered task right from the start. Additionally, Weed et al. (1999) trained NHPs on all tasks concurrently, whereas we trained NHPs on a single task at a time, allowing the NHPs to focus on learning one task rule set at a time. The SOSS training times presented here are an average of all NHPs trained to date, but optimization over time resulted in even shorter training times for the most recent NHPs trained. This is evidenced by the clustering of NHPs 6–9, which were the last to be trained, near the quicker end of the training time plots (Fig. 7B, D, and F), suggesting that our average training values most likely under-estimate how quickly NHPs can be fully trained. In addition, examination of the composite training curves of all the NHPs suggested that there was still room for further refinement of the method because the 2-box condition showed no erosion of performance when moving across the more difficult task variations. This implies that NHPs were not gaining value from the intermediate steps between the white box variation and the unaltered variation, and going forward, it should be possible to skip the intervening light blue and darker blue stimuli, thereby reducing training times by a further 4–5 sessions.

Using our progressive shaping method to teach the DMTS to NHPs required an average of 19.2 training sessions to reach a stable performance above criterion (> 60% correct on all delays using 3 distractors and the less distinguishable stimuli set). Training times presented here were far faster than previously reported values for DMTS training times. For instance, in one study, female cynomolgus monkeys required 3 months of DMTS training to learn a two-distractor version of the task using a stimuli set of only 6 different stimuli (Kromrey et al., 2015a; Kromrey et al., 2015b). Although the delays being used in their study were longer (30–60 s) than what we used (max 16 s), the performance criteria to establish whether a NHP was trained was significantly lower (50–75% correct) than ours (> 80%) (Kromrey et al., 2015a; Kromrey et al., 2015b). In another study, male rhesus macaques trained on a Delayed Non-Match-To-Sample (DNMTS) task using the same stimuli set as the difficult to distinguish stimuli set we used required an average of 63.5 training sessions (Weed et al., 1999). Although it may be problematic to directly compare DMTS and DNMTS results, both versions of the task rely on the same principles of object differentiation and...
visual memory.

The methodology we used to train our NHPs on the PAL task was very similar to that used in Taffe et al. (2004). Both studies started with the 1-stimuli PAL conditions before subsequently introducing more distractors and then the 2- and 3-stimuli conditions. Despite the similar approach to PAL training, we were able to achieve performance criteria in our NHPs within approximately 55 training sessions whereas it appears to have taken them approximately 125 training sessions to teach PAL to the same level. This discrepancy could be due to the fact that we trained our NHPs on one specific task at a time, whereas Taffe et al. trained their NHPs on many different tasks in each session, as well as the fact that our NHPs had already learned the SOSS tasks and DMTS tasks prior to starting PAL training. This sequential training paradigm we employed could have helped our NHPs learn the PAL task quicker, because spatial working memory and visual working memory concepts were already taught through SOSS and DMTS task training, respectively, and therefore the framework to learn the PAL task, which incorporates both of these memory components (Owen et al., 1995), was already in place.

4.2. CANTAB as a translational research tool

One of the steps needed to compare animal models of disease with the human diseases they are intending to emulate is a common method of measurement (Al Dahhan et al., 2019). In order to accurately make cross-species comparisons of cognitive functions and their impairments, the cognitive tests employed must target the same neural mechanisms. The degree to which a cognitive test can probe the same cognitive processes across different species determines how translationally valid that tool is (Keeler and Robbins, 2011), and it is within this context that the usefulness of the CANTAB emerges. The language free touch-screen nature of the CANTAB allows for researchers to use the same battery of neuropsychological tests they would in humans as with NHPs or rodents, allowing comparisons of cognitive performance across species.

Primary validation for the CANTAB comes from the qualitatively similar cognitive performance profiles of healthy humans and NHPs on the various CANTAB tasks. For instance, both healthy humans and NHPs show reduced performance with increasing box number in the SOSS task, made more errors during the intra-extra dimensional set shift task, and made more errors on the DMTS task at longer delay durations (Weed et al., 1999). Further validation of the cross-species capabilities of the CANTAB comes from studies showing that human patient populations and the NHP models seeking to recapitulate them show similar cognitive impairment patterns. Multiple studies have shown that both Manganese-treated and MPTP-treated NHP models of Parkinson’s disease (PD) and human PD patients display similar task-specific cognitive deficits (Schneider et al., 2015; Schneider, 2006; Schneider et al., 2013). A mouse model of Huntington’s disease (HD), caused by knocking in Q175, showed severely attenuated learning curves in the PAL task similar to what is observed in human HD patients (Piopponni et al., 2018), and a rodent Dlg2-deletion model, a gene implicated in Schizophrenia, showed cognitive deficits similar to what is found in human Schizophrenia patients (Nithianantharajah et al., 2013; Bussey et al., 2013). In addition to disease models, there are also a number of studies examining the effects of various pharmaceutical agents on CANTAB performance in NHPs with findings that show similar cognitive profiles to their human counterparts. For example, administration of the amnesiac: scopolamine (Taffe et al., 1999) and ketamine (Taffe et al., 2002b) produced altered cognitive function in keeping with what is observed in humans (Curran and Morgan, 2000; Robbins et al., 1997).

Another strength of the CANTAB is the fact that there already exists large normative data sets for individual human patient populations, including AD (Swainson et al., 2001; Junkkila et al., 2012; Égerházi et al., 2007), mild cognitive impairment (Swainson et al., 2001; Blackwell et al., 2004), HD (Lawrence et al., 1998; Lawrence et al., 1996; Lange et al., 1995), and PD (Owen et al., 1993; Morris et al., 1988; Lewis et al., 2003), allowing for the direct comparison of any developed animal disease model to this pre-existing CANTAB database. This facilitates reverse translational opportunities and the ability to generate models that match the cognitive template of human diseases. For instance, the PAL and DMTS task performance have been shown to be sensitive at discriminating pre-clinical MCI from AD (Swainson et al., 2001; Blackwell et al., 2004), suggesting PAL and DMTS performance correlates with disease severity and progression, as well as being predictive of whether individuals would go on to develop AD (Chen et al., 2000). It is because of their sensitivity in revealing deficits in human patients with AD and other neurodegenerative diseases that the SOSS, DMTS, and PAL tasks were chosen for use with our NHPs (Swainson et al., 2001; Junkkila et al., 2012; Égerházi et al., 2007; Fowler et al., 2002; Lee et al., 2003).

4.3. Comparison of reward paradigms

Comparison of the differences in training times between NHPs that were water-restricted and those that were food-delayed did not reveal any statistically significant differences. However, water-restricted animals trended towards acquiring the tasks at a quicker pace than their food-delayed counterparts. This finding is in keeping with what we have noticed anecdotally, that water-restricted animals tend to be more motivated to complete trials and as a result, we have moved to only using water-restriction during the training of our NHPs on CANTAB tasks. It should be noted though that some NHPs we have encountered have been unable to learn to accept liquid rewards from the sipper tube and were therefore trained using a food-delayed approach. We were unable to evaluate any sex differences in task acquisition as we only had a single female in our sample.

4.4. Conclusions

In summary, here we present a training methodology for teaching NHPs a battery of CANTAB tasks (SOSS, DMTS, and PAL) with the explicit goal of reducing training times so that probing NHP cognitive function via the CANTAB can be used more effectively as a translational experimental tool. Reducing training time of NHPs on the CANTAB is a necessity to permit high throughput experiments to validate models of neurodegenerative diseases and test the effect of potential therapeutics on learning and memory. We show that using a training paradigm that utilizes behavioral shaping and sequential task learning, significantly faster training times than previously published may be achieved. Overall, it is our hope that by optimizing the training of NHPs on these tasks, future research can incorporate the probing of CANTAB task performance in NHP models of neurodegenerative diseases, thereby providing directly translatable cognitive correlates between NHP disease models and human populations.

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Declaration of Competing Interest

All authors have no interests to report.

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Appendix A. Supplementary data

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