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Visual Discrimination Training for Rats: Developing a New Methodology to Explore Laterality Differences

by

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Honors Thesis in Psychology
University of Richmond
Richmond, VA
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Advisor: Dr. Cindy Bukach
Abstract

The goal of this study was to examine right hemisphere specialization for faces at the neuronal level. Research has shown that facial recognition relies on the right anterior temporal lobe and involves integrating multiple features (Bukach, Gauthier, & Tarr, 2006). Evidence from rat studies confirms that the anterior temporal lobe is involved in integrating multiple object features (Eacott, Machin, & Gaffan, 2001). However, these studies did not examine differences between the brain’s right and left hemispheres. It was hypothesized that the right anterior temporal lobe is more important for feature integration. The current study aimed to develop a methodology for training rats on visual discrimination in such a way that we could test this hypothesis. Rats were trained to discriminate between faces that differ on only one feature (eyes or mouth) or on multiple features (eyes and mouth) using touch screen technology. Training was broken down into levels, and rats had to complete all levels of training with at least 80% correct responses at each level for the paradigm to be considered successful. Following development of a successful training paradigm, we plan to use immunohistochemistry techniques to examine laterality differences in neural activity. We expect to find that rats in the multiple feature group will show more neural activity in the right anterior temporal lobe than in the left. These results will confirm the importance of the right hemisphere in integrating multiple object features during face recognition and will provide the first evidence of this at the neuronal level.
Visual Discrimination Training for Rats: Developing a New Methodology to Explore Laterality Differences

People are constantly looking at the world around them and identifying their surroundings in order to accomplish even the simplest of everyday tasks. Jane can determine that the large piece of machinery in the driveway is her new BMW that she can drive to work in, and John knows that the person he just kissed goodnight is without a doubt his wife of five years. Even toddler Jake can recognize that the new creature living in the house is a dog; it looks just like what he saw in his storybook. Many theories have been created to try to explain how the human brain is able to perceive incoming information about a person's surrounding, categorize and store any new information, and recognize that which has already been stored. Indeed it is a remarkable feat. Consider the frustration that might arise if one was not able to identify a car in the driveway as a car, much less a BMW. Or what if one could not recognize his or her own family member, spouse even?

Patients with category specific agnosia experience such problems on a regular basis.

Category specific agnosia is a breakdown of object knowledge following neurological damage to the brain. Generally, patients with this impairment have agnosia for either living or nonliving things resulting from damage to one or both of the temporal lobes. Some individuals with damage to the right hemisphere have a rare form of this unfortunate impairment in which faces are the predominant type of stimuli affected. This category specific agnosia for faces is called prosopagnosia. Most people with prosopagnosia have damage to the fusiform face area (FFA), which is located in the medial inferior temporal lobe and is dominant in the right hemisphere. This area has been shown to respond strongly to faces and other stimuli that require perceptual differentiation and for
which a person has developed expertise (Gauthier, Skudlarski, Gore, & Anderson, 2000). Less well studied is damage to the right anterior temporal lobe, which also causes prosopagnosia (Bukach et al., 2006). Whether faces are special is a topic of debate, but clearly there must be something that lends them vulnerable to right hemispheric damage.

Recognition of living things and faces requires integration of complex visual information, but research on human patients suggests that the nature of visual integration may vary between hemispheres. Patients with category specific agnosia for living things cannot integrate structural dimensions (tapering, pinching, curvature) of simple geometric forms, and instead rely on a single structural dimension in a recall task (Arguin, Bub, & Dudek, 1996). In contrast, prosopagnosic patients with anterior right hemisphere damage cannot integrate multiple face features (eyes, nose, and mouth), and instead rely on a single feature (the mouth) to make identification judgments (Bukach et al. 2006). This deficit is seen in nonface objects, as well. Thus, the left hemisphere may be important for integrating the structural dimensions of geometric forms, whereas the right hemisphere may be important for integrating multiple features into a perceptual whole. This conceptualization would be consistent with left hemisphere specialization for single feature analysis and right hemisphere specialization for holistic processing.

Evidence from both humans and animals supports the role of the Perirhinal Cortex (PRh) in visual discrimination and memory of complex conjunctions. Imaging studies of humans show that anterior regions of the temporal lobe, including the PRh, become recruited when tasks require finer grained representations of objects (Tyler, Stamatakis, Bright, Acres, Abdallah, Rodd, & Moss, 2004). Patients with damage to anteromedial temporal lobes (See figure 1) are impaired when making fine-grained discriminations that
require a combination of multiple features (Barense, Bussey, Lee, Rogers, Davies, Saksida, Murray, & Graham, 2005). Similarly, damage to the PRh of the macaque disrupts the integration of complex visual features. Macaques with a lesion to the perirhinal cortex performed poorly on tasks requiring them to differentiate between pictures with high feature ambiguity, but not between pictures with low feature ambiguity. For high feature ambiguity tasks, the macaque could not simply track a single feature, but instead had to take all features of each picture as a whole (Bussey, Saksida, & Murray, 2003). Damage to the PRh of the rat (See figure 2) also disrupts perceptual discrimination of compound stimuli. When presented with two-choice visual discrimination tasks with stimuli of varying complexity, rats with a lesion to the perirhinal cortex could perform the basic tasks, differentiating on the basis of one feature, but could not perform the conjunction tasks, differentiating on the basis of more than one feature (Eacott, Machin, & Gaffan, 2001).

Together, these studies support the notion that across species, object recognition is organized in a hierarchical fashion, with the complexity of object representations increasing as the visual stream moves towards the anterior temporal lobe (Bussey & Saksida, 2005). However, in all of these studies, damage to the perirhinal cortex was bilateral, and so it is unclear whether compound discriminations rely equally on the right and left perirhinal cortex.

The case of prosopagnosic patient L. R. provides strong evidence that the right hemisphere is indeed relied on more when it comes to compound discriminations. L. R. suffered damage to the right anterior and inferior temporal lobe. Bukach and colleagues found that the three factors associated with face recognition, fine-level discrimination,
holistic processing, and configural processing, were still functional in L. R. (2006).
However, his application of these processes were limited spatially. L. R. could differentiate
facial features only for the lower half of faces. In other words, he could not integrate
multiple features of a face in order to correctly identify the person.

The present study will attempt to reveal whether a procedural difference in the
abilities of the right and left temporal lobes exists by extensively training rats to make
discriminations based on either one feature or based on multiple compound features and
then looking for differences in right and left neuronal activity of the perirhinal cortex. The
animal studies mentioned above all used lesion methods, in which the brain areas of
interest were damaged. The present study will use immunohistochemistry techniques
instead, which will show evidence of recent neuronal activity following training upon
examination of the brain. This would provide converging evidence for hierarchical
organization, as well as extend these studies to examine laterality differences.

Current research for this study has focused on developing a methodology for
training the rats to successfully discriminate among face-like stimuli using touch screen
technology. Touch screens have been shown to be successful training tools for rats for
discrimination tasks (Markham, Butt, & Dougher, 1996) and to support faster acquisition of
visual discrimination abilities in rats (Cook, Geller, Zhang, & Gowda, 2004). By using face-
like stimuli, results can be compared more easily to those of human studies such as Bukach
et al. (2006). In addition, by using the same four stimuli in all conditions, this study
provides better control of stimuli than that of Eacott, Machin, and Gaffan (2001), who
employed different stimuli for the basic tasks than for the conjunction tasks, yet will keep a
very similar training paradigm. This study is an important step in investigating laterality
differences in feature integration. Potentially, we will be able to learn what contributes to right hemisphere specialization for face recognition and utilize that knowledge in order to aid the development of treatment for prosopagnosic patients.

Method

*Hypothesis:* Rats in the multiple feature group will show more neuronal activity in the perirhinal cortex of the right anterior temporal lobe than in the left. There will be no laterality difference in the perirhinal cortex for the single feature group or for control regions (hippocampus).

*Current Goal:* A working methodology for training rats to discriminate among face-like stimuli must be developed in order to test the hypothesis. This is the goal of the present research.

*Version 1*

*Subjects:* 4 male albino Fisher rats were randomly assigned to groups (single feature group=2; multiple feature group = 2) for Version 1 of this study. Rats were 6 months old at the start of the experiment. They were housed 2 per cage and kept on a 12 hour light- 12 hour dark schedule. One of two trainers trained rats during their dark cycle in a dark room. The single feature group was trained to discriminate using only one feature of the face-like stimuli (i.e. eyes or mouth), and the multiple feature group was trained to discriminate using both features (i.e. eyes and mouth). By training the two groups on separate types of discrimination, we hope to later be able to test our hypothesis by comparing enhancement and signs of activity in the brain areas of interest. All rats had unlimited access to water, but were food deprived to 85% of their free-feeding weights. Weights were recorded daily prior to training.
**Materials:** Stimuli measured 4cm in diameter and were black with a white background. The pretraining stimulus was a black outline of a circle. Training stimuli consisted of 4 face-like cartoons that have two varying features (See figure 3). Conjunction stimuli were created using every possible combination of the two eyes and two mouths. Two of the faces were targets. Targets for the single feature discrimination group were matched with a distractor in such a way that attention to only one feature (the eyes or the mouth) is needed. Targets for the multiple feature discrimination group were matched with a distractor in such a way that attention to both the eyes and the mouth is necessary.

An Entuitive Touchmonitors touch screen, measuring 25.5cm x 19cm, was used to present the experiment, which was programmed in SuperLab and run on a Macintosh laptop. Rats were trained in a 27.5cm long x 21cm wide x 19.5cm tall cage with wire flooring and clear plexiglass walls. One-fourth of a Froot Loop cereal piece was used as a food reward.

**Training Methods:** Rats received extensive training on visual discrimination of stimuli over the course of several weeks. Each rat participated in one training session per day, 5 days per week. Training sessions ran through 101 trials or were ended after 1 hour. Trials were initiated by the trainer with a push of the laptop’s space bar. A tone signaled the initiation of the trial to the rat. Rats were given an unlimited amount of time to respond. The rats were rewarded with a small edible treat after each correct response (Cook, Geller, Zhang, & Gowda, 2004). A ringing tone signaled a correct response. The trainer promptly dropped the reward into a hole in the rear wall of the cage, and rats were given time to finish the food before the next trial was initiated. Incorrect responses were followed by a flashing cross on the screen and a brief pause before next trial could begin.
Before discriminating between stimuli, rats first completed pretraining in which they were taught to use the touch screen and to touch a target object. During the first pretraining phase (Any Touch or AT), a white circle appeared on either the right or left side of the screen. Rats were simply required to touch anywhere on the touch screen. AT was completed when the rat had completed 80 trials with a successful touch during one session.

During the second phase of pretraining (Target Touch or TT), rats were required to touch the side of the screen (left of y axis or right of y axis if on a coordinate plane) on which the white circle appeared in order to be rewarded. This phase was complete when the rat reached at least 80% accuracy.

After pretraining was complete, rats moved on to Phase Level 1, which consisted of Steps A, B, C, and D (See table 1 for training schedule). Phase Level 1 was the first to use the face-like stimuli and to require discrimination between two stimuli. For Steps A and B, stimuli were paired in such a way that rats in the one-feature group could discriminate their targets from the distractors by looking at either the mouths or the eyes depending on their assigned condition. The two-feature group had to discriminate first by using one feature, then by using the other feature for their target. Steps C and D trained rats on a second target in the same manner. Each step was completed when rats reached the 80% or higher accuracy criterion.

Phase Level 2 alternated Steps A and B between days until criterion was reached for both steps in two consecutive days (Step E). Then, Steps A and B are randomly combined into one session until 80% criterion is reached. The same procedure is then used for steps C and D (Step F). Phase Level 3 alternates Steps E and F between days. For completion of this phase, rats must achieve 80% criterion on two consecutive days. Finally, Phase Level 4
randomly combines steps E & F so that now the original Steps A-D are all displayed in a single final session.

*Results:* Under Version 1, rats took an average of 2.75 days to complete AT and 5.5 days to complete TT (See figure 4). Unfortunately, all rats were unsuccessful at completing SA after 5 to 7 training sessions. Furthermore, during those sessions, no rat scored significantly above chance (all scores ranged from 39% to 57%, where 50% was the expected accuracy if responses were given only by chance). It was suggested that this may have been because the rats could very quickly go from the back of the cage where they received their reward to the touch screen without much time in between to actually look at what was on the screen. Version 2 was created as an attempt to make the rats focus on the actual stimuli.

**Version 2**

*Subjects:* 8 male albino Fisher rats (single feature group=4; multiple feature group = 4) were used in version 2 of this study, including the 4 used in Version 1. Rats were now 9 months old at the beginning of version 2. Previously designated groups and experimental conditions were never changed. New rats were randomly assigned to conditions. Rats were now trained during their light cycle in a light room by one of two trainers. All rats maintained a restricted diet and weights that were 85% of their free-feeding weights.

*Materials:* Stimuli colors were inverted; stimuli were now white with a black background. The pretraining stimulus was changed for a black outline of a circle to a white filled in circle. The original cage was doubled in length (from 27.5cm long to 55cm long) (See figure 5). All other materials were kept the same.
Training Methods: A few changes were made in Version 2. The cage was made twice as long. This increased the distance rats had to travel from the back of the cage where they received their reward to the front where the touch screen was located and forced rats to look at the screen for a longer amount of time as they approached it. Also, the rats were now required to touch the actual 4cm diameter target stimulus rather than just the side of the screen on which it appeared. If they touched the correct side, but did not touch the target itself, the response was counted as incorrect.

Results: It was thought that these changes would teach the rats to pay more attention to the stimuli themselves. However, even with these changes, rats were unable to complete SA after an average of 8 training sessions. We hypothesized that rats needed to learn an easier discrimination task before they would be able to perform discrimination tasks for the face-like stimuli. Also, TT took longer to complete, taking an average of 6.1 training sessions (See figure 6) compared to an average of 5.5 training sessions under version 1. This was likely because rats had more difficulty touching only on the target stimulus, especially when their whiskers span a length greater than that of the stimuli.

Version 3

Subjects: 8 male albino Fisher rats (single feature group=4; multiple feature group = 4) were used in version 3 of this study. They consisted of the four that were used in Version 1 along with four new rats. Rats were now 10 months old. Again, previously designated groups and experimental conditions were never changed. New rats were randomly assigned to conditions. Rats were trained by one of two trainers. Weights were still maintained at 85% of free-feeding weight.

Materials: All materials were kept the same.
Training Methods: A pre-step to SA was added (SA1). SA1 paired the target face-like stimulus, which varied depending on experimental condition, from SA with the plain circle from TT. The four newest rats started training with AT. The four rats from Version 1 started Version 3 with the new pre-step SA1. In the added SA1 phase, a face-like stimulus appeared on one side and the white circle from TT appeared on the other. Rats were now required to touch on or very near the target stimulus (within 1.5cm of the stimulus circumference). It was believed that jumping from TT to actual discrimination between the face-like stimuli was too great a leap for the rats. Also, it was believed that having to touch the stimulus itself without touching any other part of the screen was too difficult for the rats, whose whisker span exceeded the width of the stimuli. To remedy this, for all phases following AT, touching on or very near the target stimulus was now counted as a correct response. Also, if one side of the screen was touched, but neither stimulus itself was, this was no longer counted as incorrect; it simply did nothing.

Results: The newest rats that had started with AT took an average of 2.75 days to complete AT and an average of 3.5 days to complete TT (See figure 7). This was the fastest that rats had passed both pretraining steps. Still, rats did not seem to be learning the discrimination tasks. No rat completed the new pre-step. We hypothesized that using the white circle, which had been the target during TT, as the distractor during SA1 may have been throwing the rats off. A different stimulus was needed for SA1. Also, it would be good to make sure that the rats can discriminate between visual stimuli at all. We decided to add a discrimination pretraining task that the rats should be able to successfully complete based on previous studies. In a 1975 study, rats were successfully trained to discriminate
between vertical and horizontal lines (Krivanek). This simple discrimination might provide a necessary bridge between the pretraining and the discrimination tasks.

**Version 4**

*Subjects:* Only the 4 rats that had been used in version 1 were used in Version 4 of this study (single feature group=2; multiple feature group = 2). Rats were now 1 year old. Rats were trained by one of three trainers.

*Materials:* A vertical-horizontal discrimination task using non face-like stimuli (VHL) was added prior to SA1 using two new stimuli. One was a circle filled with black and white vertical lines. The other was a 90 degree rotation of the first creating a circle filled with black and white horizontal lines. Each circle had a 4cm diameter with lines that were approximately 7mm thick. The distractor stimulus for SA1 was changed from a white circle to circle with three asterisks inside. The asterisks were positioned where the face-like features would have been (See figure 8).

*Training Methods:* All rats started from the very beginning with the AT phase because they had not been trained in nearly 2 months due to a summer break. In the added VHL phase, rats were given a discrimination task that was known to be learnable after TT and before SA1. In this phase, the circle with vertical lines appeared on one side of the screen and the circle with horizontal lines appeared on the other. The target was always the circle with the horizontal lines. VHL preceded SA1, which was changed from a discrimination between a face-like stimulus and a white circle to a discrimination between a face like stimuli and a circle with asterisks inside.

*Results:* Pretraining took only 3 days for both AT and TT to be completed (See figure 9). However, the rats used in this version had already been trained in all of the previous
versions. Only one of the four rats completed VHL, taking 5 sessions to do so. He moved on to SA1, but did not complete that phase. The other three rats did not complete the VHL phase in an average of 9 to 11 training sessions. It was thought that the rats might need exposure to the target stimulus earlier in the training paradigm, prior to making this discrimination.

**Version 5**

*Subjects:* The 4 rats that had always been used in versions 1 through 4 were used in version 5 of this study (single feature group=2; multiple feature group = 2). Rats were now 13 months old. Rats were trained by one of four trainers.

*Materials:* All materials were kept the same.

*Training Methods:* Horizontal Target (HT) was added in Version 5 after the original Target Touch. The rat that had completed VHL under Version 4 was not redirected back to HT. He continued on to SA1. The other three rats started Version 5 with HT. The added HT phase was identical to TT except it used the horizontal line stimulus instead of the white circle in order to expose rats to seeing it as a target before attempting VHL. In Version 5 of the training paradigm, the phase order for training was: AT, TT, HT, VHL, SA1, SA (See table 2 for the order of training phases under each version).

*Results:* The rat that started Version 5 with SA1 passed in 3 days. Unfortunately, this rat died due to unknown causes before continuing on further. The rats put on HT passed in an average of 3.7 training sessions (See figure 10). However, none of these rats managed to pass VHL in 6 to 8 training sessions. At this point, it came to our attention that albino rats, like those we were using, have extremely poor visual acuity (See figure 11)(Whishaw & Kolb, 2005). We also noted that the rats might have been associating the reward too much.
with the trainer rather than with giving a correct response. To remedy this, an automatic pellet dispenser would be ideal.

**Version 6**

*Subjects:* 3 male Long Evans rats were randomly assigned to groups (single feature group=2; multiple feature group = 1) for Version 6 of this study. Long Evans rats were chosen because their visual acuity is superior to that of the albino Fisher rat, and our lab was able to acquire them in a timely manner. Long Evans rats have grating thresholds around 1.0 cycle/degree while albino rats have grating thresholds around 0.5 cycle/degree (Prusky, Harker, Douglas, & Whishaw, 2002). Rats were housed individually, and were kept on a 12 hour light- 12 hour dark cycle. Rats were trained by one of four trainers during their light cycle in a light room. A restricted diet was used to maintain rats at 90% of their free-feeding weights.

*Materials:* Only one pretraining stimulus was used in Version 6. The white filled-in circle was used in AT and TT. The face-like stimuli were kept as well for the training stimuli. However, the curved mouth (M1) was inverted to make it even more different from the pointed mouth (M2) (See figure 12). An automatic pellet dispenser was employed to administer a sugar pellet as a reward. Following a correct response, the trainer could press a handheld button that would trigger the release of a pellet into a holder in the back of the training cage.

*Training Methods:* Programming was changed so that trials would advance automatically after response input. All rats began with AT. The added pre-steps in previous versions were taken out. Markham and colleagues found that Long Evans rats were able to make discriminations between simple shapes, like those that make up our face-like stimuli,
after completing a pretraining that was very similar to our AT and TT phases (1996). In Version 6, the phases were ordered: Any Touch (AT), Target Touch (TT), Target Touch with Incorrect Response Option (TTwIR), and then the face-like discriminations. For TT, rats were rewarded for touching the target, and if they touched anywhere else on the screen, nothing happened. For TTwIR, rats were rewarded for touching the target or the area within 1.5 cm of the circumference of the stimulus. If they touched the side of the screen that the target stimulus was on, nothing happened. If they touched the side of the screen that the target was not on, an incorrect response was recorded. For this version, there were no sounds except for a brief tone after an incorrect response.

Discussion

Our choice of rats for the first 5 versions was very poor. We failed to take into consideration the rats’ visual acuity. The albino rats likely could not pass the tasks requiring visual discrimination because they could not see well enough to make the discriminations. About a year and a half into the study, we came across a 1996 study that was very similar to ours conducted by Markham, Butt, and Dougher. They too had started their training using albino rats. When the albino rats were not successfully learning, the researchers switched to Long Evans rats. Training with the Long Evans rats was successful. By following their lead, and using Long Evans rats, we hope that Version 6 will be successful.

We have found that a touch screen can be very a very useful training tool. However, it needs to be used correctly. When using a touch screen to train rats on visual discrimination, it is important to consider several factors that may affect your experiment. First, the rats must have the visual acuity needed to see the chosen stimuli as they are
presented on the monitor. Rats will need some sort of pretraining to introduce them to using the touch screen before jumping into discrimination tasks. Also, it is important to remember that rats greatly rely on their sense of smell and that the touch screen should be cleaned between training subjects.

The current study is still working to develop a paradigm that will successfully train rats to make visual discriminations for face-like stimuli using a touch screen. A successful training paradigm will allow for the exploration of laterality differences in neuronal activity following discrimination training. Theoretically, the Long Evans rats should be able to learn to discriminate among our face-like stimuli. The simple shapes used by Markham et al. (2006) are similar to those that make up the features of our stimuli. We hope that the difference in visual acuity between the old albino Fisher rats and the current Long Evans rats will account for the failed previous attempts at developing a training paradigm. Ideally, Fisher-Norway rats would be used for this type of visual discrimination, because their visual acuity is even better than that of the Long Evans (Whishaw & Kolb, 2005). Fisher-Norway rats were unattainable for the current study, but may be a worthwhile possibility in the future.

This study’s future directions can further define the function of the anterior temporal lobe with respect to cognitive processes necessary for facial recognition in terms of laterality differences. Once a successful paradigm is developed, immunohistochemistry can be used to reveal evidence of recent neuronal activity in areas of interest in the brain, namely the Perirhinal Cortex, after discrimination training. C-fos techniques have been suggested, but there are other techniques that demand consideration. Future
experimenters will need to research further to find the best immunohistochemistry technique for testing our hypothesis.
References


Figure Caption

*Figure 1.* Human brain with anteromedial temporal lobe area shaded.

*Figure 2.* Rat brain with perirhinal cortex area shaded.

*Figure 3.* Face-like cartoon stimuli used for visual discrimination training in Versions 1-5.

*Figure 4.* Chart showing the average number of training session days needed to complete training phases under Version 1. AT and TT averages based on 4 rats.

*Figure 5.* Photograph of training chamber with touch screen used for Versions 2 through 6.

*Figure 6.* Chart showing the average number of training session days needed to complete training phases under Version 2. AT average based on 8 rats. TT average based on 7 rats.

*Figure 7.* Chart showing the average number of training session days needed to complete training phases under Version 3. AT average based on 4 rats. TT average based on 2 rats.

*Figure 8.* Asterisk stimulus used as a distractor for SA1, where the target was a face-like stimulus.

*Figure 9.* Chart showing the average number of training session days needed to complete training phases under Version 4. AT and TT averages based on 4 rats. Number of days for VHL completion based on only one rat.

*Figure 10.* Chart showing the average number of training session days needed to complete training phases under Version 5. HT based on 3 rats. L1SA1 based on only one rat.

*Figure 11.* The degree of blurring seen by different rat strains compared to that of humans. Note the reduced contrast of heavy black lines for albino rats (Whishaw & Kolb, 2005, p. 53).
Figure 12. Face-like cartoon stimuli used for visual discrimination training in Version 6.
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Version 3

Training Until Phase Completion (Days)

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Visual Discrimination Training 29
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Table Caption

*Table 1.* Training schedule (Assignment of objects to target and distractor conditions were counterbalanced across subjects)

*Table 2.* Order of training phases for each version
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<td><strong>Level 2</strong></td>
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<td><strong>Step E</strong></td>
<td>E1M1+ vs. E1M2- and E1M1+ vs. E2M2</td>
<td>E1M2+ vs. E1M1- and E1M2+ vs. E2M2-</td>
</tr>
<tr>
<td><strong>Step F</strong></td>
<td>E2M1+ vs. E1M2- and E2M1+ vs. E2M2-</td>
<td>E2M1+ vs. E1M1- and E2M1+ vs. E2M2</td>
</tr>
<tr>
<td><strong>Level 3</strong></td>
<td>Step E &amp; F are alternated between days until 80% criterion is reached on 2 consecutive days.</td>
<td></td>
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<tr>
<td><strong>Level 4</strong></td>
<td>All four trial types are randomly combined in a single final session.</td>
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</tr>
<tr>
<td>Version</td>
<td>Order of Training Phases</td>
<td></td>
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<tr>
<td>1</td>
<td>AT, TT, Face-like stimuli discriminations</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AT, TT, Face-like stimuli discriminations</td>
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</tr>
<tr>
<td>3</td>
<td>AT, TT, L1SA1, Face-like stimuli discriminations</td>
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<tr>
<td>4</td>
<td>AT, TT, VHL, L1SA1, Face-like stimuli discriminations</td>
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<tr>
<td>5</td>
<td>AT, TT, HT, VHL, L1SA1, Face-like stimuli discriminations</td>
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</tr>
<tr>
<td>6</td>
<td>AT, TT, TTwIR, Face-like stimuli discriminations</td>
<td></td>
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