Transitive Inference in Rats Using Odor Stimuli: Manual Versus Automated Training Procedures

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ABSTRACT
Transitivity is a type of higher-order learning that is demonstrated when untrained relations emerge after specific relations have been trained. The formation of a hierarchy is required to determine the transitive relations between stimuli. To train the hierarchy A<B<C<D<E, the pairs A-B$^+$, B-C$^+$, C-D$^+$, and D-E$^+$ are presented (the plus sign indicates the reinforced stimulus). After training, novel pairs, such as A-C$^+$ and B-D$^+$ are presented to test for transitive inference. This study compares three rats' performances on tests of novel pairs in a manual procedure and four rats' performances in an automated procedure. While automated procedures generally increase efficiency and objectivity and are a preferred method to manual procedures, rats trained using the automated apparatus failed to meet baseline training criterion and were not successful at tests of novel pairs. All rats trained with the manual procedure demonstrated transitive inference. The ecological significance of transitive inference and the testing procedure is discussed.

INTRODUCTION

Concept learning is a type of higher-order learning requiring the formation of categories. Categories are formed by utilizing features, such as shape or function, to group stimuli (Lazareva & Wasserman, 2008). Perceptual concepts, nonsimilarity-based concepts, and abstract concepts are three types of concepts used to evaluate and categorize stimuli. Perceptual concepts are shown when physical dimensions of the stimuli are the basis for categorization. Nonsimilarity-based concepts include stimuli that are physically different but have a similar function (Lazareva & Wasserman, 2008).

Abstract concepts are demonstrated when the relations among the stimuli are used to categorize the stimuli in context with one another (Lazareva & Wasserman, 2008). For example, if it has been trained that stimulus A equals stimulus B (A=B) and stimulus B equals stimulus C (B=C), the presentation of stimulus A and C would create a novel situation. However, by utilizing information from previously trained pairs, a relation between the two could be made. This relation would be that stimulus A equals stimulus C (A=C) (Sidman, Rauzin, Lazar, Cunningham, Tailby, & Carrigan, 1982). The example of the relation between stimulus A and C is an example of transitivity, one of the properties of the equivalence concept.

Equivalence involves making relations about items that have never been trained together (Davis, 1992; Lazareva & Wasserman, 2006). For example, if the pairs A=B and B=C are trained during baseline training, they are grouped into one class. A class is a group of specific stimuli that are interchangeable (McIntire, Cleary, & Thompson, 1987). Training that classifies stimuli A, B, and C as one class allows novel probe tests to be given to test the relations that have been formed between the stimuli. The emergence of untrained relations between novel pairs such as reflexivity (A=A, B=B, C=C), symmetry (B=A, C=B), and transitivity (A=C, C=A) represents equivalence; the stimuli have never been presented together or in that order, yet subjects may respond as if the stimuli are related (Sidman, 2000). Nonhumans do not typically show the formation of all equivalence relations. In fact, the formation of equivalence relations has been considered a form of higher-order learning that requires the use of language (Hayes, 1989). However, Schusterman and Kastak (1993) provided the first concrete evidence that, given extensive training, a nonhuman animal (a sea lion) could form all equivalence relations.

Schusterman and Kastak (1993) trained a California sea lion on 30 equivalence classes in order to provide multiple exemplars. Each class was composed of three stimuli (A, B, and C). The first phase of training involved presentations of stimuli A_1-A_{30} as samples and B_1-B_{30} as comparisons. An example of this training is presenting A_1 as the sample and B_1 and B_X as comparisons. Responses to B_1 were reinforced and responses to any other B stimulus (B_X) would not result in reinforcement. After this training, 12 of the A=B pairs were tested and then trained for symmetry (B=A). Not until the sea lion performed at a high level on presentations of B=A did the training of B=C begin. Once the sea lion met criterion on the B=A training, 30 B=C pairs were trained. Then, the same 12 pairs that were used to test B=A were used to test and train symmetry with the B=C pairs. After this symmetry training (C=B), the same 12 potential equivalence classes were tested and trained for transitivity (A=C). Following this training, those 12 potential equivalence classes were tested for C=A symmetry. This extensive training provided multiple exemplars for the sea lion before the final test of equivalence was conducted.

A higher-order learning task that involves the presentation of novel pairs of stimuli to determine the relations that have been made is a test for transitive inference. In a typical procedure to test for transitive inference, a hierarchical order of the stimuli is trained. For example, given the two statements "Alex is shorter than Brad" and "Brad is shorter than Chris," the hierarchy in terms of height would be Alex<Brad<Chris. The inference that Alex is shorter than Chris can be determined due to their relationships to Brad in the hierarchy.

The training of a hierarchy is done by presenting pairs of stimuli and always reinforcing responses to one stimulus and never reinforcing responses to the other stimulus (Lazareva et al., 2004). For example, given the three stimuli A, B, and C, the hierarchical order A<B<C can be trained. This can be done by presenting two pairs, A-B and B-C. In the pair A-B, responses to B are always reinforced and responses to A are never reinforced. With the pair B-C, responses to C are always reinforced and responses to B are never reinforced. Once the training of the hierarchical order is complete, the test for transitive inference can be presented. This involves the presentation of the novel pair A-C. Using the trained hierarchy, a relation between stimulus A and stimulus C can be made (A<C). Though information relating stimulus A and C has not been directly trained, a relation between the two stimuli can be inferred due to their relation to stimulus B (A<B<C) (Davis, 1992).

Though a hierarchical order can be established using three stimuli, the novel pair (A-C) does not represent a true test of transitive inference. Stimuli A and C are called end stimuli, and success on a novel pair containing end stimuli can be explained by reinforcement histories (Dusek & Eichenbaum, 1997). Therefore, a minimum of a five-item list is needed to ensure that the novel test pair does not include an end stimulus (Weaver, Steirn, & Zentall, 1997). In a five-item list (A, B, C, D, and E), training would occur by presenting the following pairs: A-B, B-C, C-D, and D-E. Within these pairs, one stimulus would always be reinforced and the other would never be reinforced, A-B$^+$, B-C$^+$, C-D$^+$, and D-E$^+$ (the plus sign indicates the reinforced stimulus).

The five-item list allows for a true test of transitive inference because two of the middle stimuli have equal reinforcement histories and have never been presented together (stimulus B and stimulus D). Success on B-D$^+$ demonstrates transitive inference because both B and D have been previously reinforced 50 percent of the time they were presented and not reinforced the other 50 percent of the time. B and D have equal reinforcement histories and therefore, during a transitive inference test, the choice of D$^+$ indicates that a relation between stimuli has been formed (Weaver et al., 1997).

Transitive inference has been demonstrated in a number of non-human species including chimpanzees (Gillan, 1981), rhesus macaques (Treichler & Van Tilburg, 1996), hooded crows (Lazareva et al., 2004), pigeons (von Fersen, Wynne, Delius, & Staddon, 1991; Lazareva & Wasserman, 2006), and rats (Davis, 1992; Dusek & Eichenbaum, 1997). Davis (1992) and Dusek and Eichenbaum (1997) used olfactory stimuli to test transitive inference in rats. In both studies, rats have successfully performed tasks using olfactory stimuli rather than visual stimuli. Rats succeeded in the transitive inference tasks even though the olfactory stimuli were not orderable on any dimension other than reinforcement history.

Davis (1992) trained four rats with the series A-B$^+$, B-C$^+$, C-D$^+$, and D-E$^+$ to establish the hierarchy $A<B<C<D<E$. Olfactory stimuli were used, and the apparatus was a "Y" maze with a door at the end of each arm of the "Y." The doors were scented with different food flavoring. The rats were trained on the following: A-B$^+$, B-C$^+$, A-C$^+$, C-D$^+$, D-E$^+$, C-E$^+$, and A-E$^+$. Then, the novel pair, B-D$^+$ was presented, and the rats chose D over B even without training.

Dusek and Eichenbaum (1997) also used olfactory stimuli, cups of scented sand, to train rats with a five item list ($A>B>C>D>E$). Pairs were trained in five phases similar to Davis (1992). Transitive inference was tested with 10 trials of the B-D pair and 10 trials of the A-E pair. New scents (W-X and Y-Z) were also presented to measure the rate at which the rats learned novel pairs. The rats demonstrated transitive inference when tested on the B-D pair and showed some evidence for learning the novel scent pairs.

Jordan (2009) replicated Dusek and Eichenbaum's (1997) methods to train three rats on two five-item lists. She reported results from two rats and demonstrated transitive inference. In the current study, I will present results from the third rat trained with the Jordan (2009) procedure. Her procedure involved the manual presentation of all stimuli, and I will also compare her findings related to transitive inference using a manual procedure to those obtained using an automated procedure.

Success with olfactory tasks has been demonstrated with rats in automated apparatuses. Otto and Eichenbaum (1992) trained rats in a delayed non-match-to-sample task using odor stimuli that were presented through a nose poke in the operant chamber. The rats rapidly learned the task. Lionello-DeNolf and Mihalick (2006) used an automated olfactory apparatus to tests rats' ability to learn a simple discrimination task, followed by a reversal. The results of the study supported the use of an automated apparatus in olfactory tasks and suggested that more complex tasks could be tested using an automated apparatus.

The use of an automated apparatus increases both efficiency and objectivity, and therefore, is a preferred method to manual procedures. Transitive inference tasks have never been tested in an automated apparatus, and the objective of this study was to train rats to form a hierarchy with five scents and test the performance on a transitive inference task.

We expected that transitive inference would be apparent using the manual and automated apparatuses. We hypothesized that rats' performances on the B-D^+ probe would be above chance levels, and the rats would choose the D stimulus over the B stimulus. We also expected that rats would learn the B-D^+ relation more rapidly than a relation between two unfamiliar, untrained scents.

METHOD

Manual Procedure

Subjects

Three male Sprague-Dawley rats were trained using a manual procedure described by Jordan (2009). Jordan reported data from two rats (P39 and P23), and data from rat S16 is reported here. Rats were kept at 85-90% of their free-feeding weight with unlimited access to water. Approximately 30 minutes after finishing their session, the rats were fed 10-15 grams of Purina® Rat Chow. They were housed individually in a colony room that was illuminated on a 12- hour reversed light/dark cycle.

Apparatus

Testing occurred in an operant chamber with a modified front wall. The wall had a gap allowing a Plexiglas tray to be pushed into the chamber to deliver the stimuli during testing (see Figure 1).

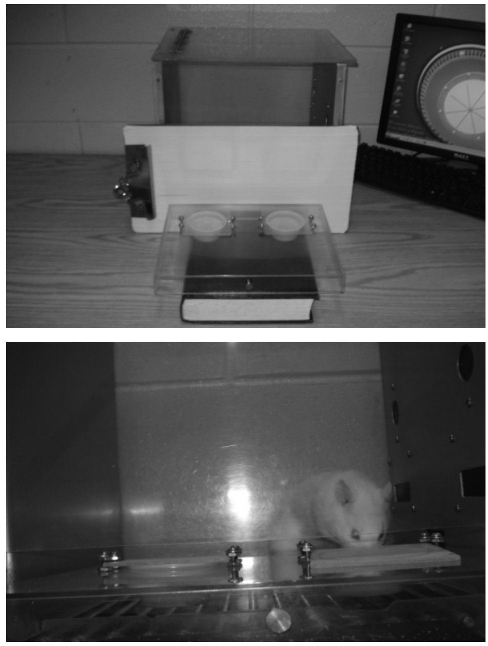

Figure 1. Manual apparatus. The operant chamber is shown in top photograph. The rat making a response is shown in lower photograph.

Stimuli were lids scented with spices purchased from the Great American and the Rocky Mountain Spice Companies. Lids were stored for at least 24 hours before use in containers with spices (caraway, marjoram, celery, mustard, allspice, lime, anise, sage, onion, paprika, raspberry, thyme, cinnamon, garlic, savory, coriander, bay, carob, Worcestershire, tomato, fennel, beet, ginger, turmeric, cumin, nutmeg, clove, and dill).

Scented lids were placed on top of 2-ounce plastic cups. A layer of sand was placed in the bottom of each cup. Sucrose pellets (.45 mg) were used as reinforcers and were buried 0.5-0.8 millimeters below the surface of the sand underneath the scented lids. The cups with scented lids on top were placed in two holes (5 cm diameter) cut in the Plexiglas tray. All housing and testing procedures were approved by the Institutional Animal Care and Use Committee.

Procedure

Shaping

The rats were first trained to dig for the sucrose pellets. The first step in this training involved putting the sucrose pellets on top of the cups containing sand. Eventually, the pellets were buried between 0.5- 0.8 mm below the surface. The rats were then trained to remove lids by pushing them off of the sand cups.

Five-Item List Training

Two five-item lists were trained as described in Jordan (2009). List one consisted of scents A-E, and list two consisted of scents F-J. Scents were presented in pairs of two ($A\text{-}B^+$, $B\text{-}C^+$, $C\text{-}D^+$, $D\text{-}E^+$, $F\text{-}G^+$, $G\text{-}H^+$, $H\text{-}I^+$, and $I\text{-}J^+$), to create a hierarchy ($A<B<C<D<E$ and $F<G<H<I<J$). In each pair a response to one scent was always reinforced and response to the other scent never reinforced. Pushing the scented lid off of the cup was counted as a response. Correct responses were reinforced with a sucrose pellet. If an incorrect response was made, the Plexiglas tray was immediately removed from the chamber. If two minutes elapsed without a response to either cup, the tray was removed from the chamber and the next trial began.

The first five-item list was trained in four phases. The first phase consisted of the presentation of each pair in blocks of six trials. Six trials of $A\text{-}B^+$ were presented, followed by $B\text{-}C^+$, $C\text{-}D^+$, and $D\text{-}E^+$, respectively. No more than two identical trials were presented in a row, and the reinforced scents were equally balanced between the left and right side. Criterion of 80% correct on each trial type for two consecutive days was required before proceeding to the following phase. In phase two, three trials of $A\text{-}B^+$ were presented followed by three trials of $B\text{-}C^+$, then $C\text{-}D^+$, and $D\text{-}E^+$. This sequence was repeated for a total of 24 trials. Six blocks of four trials were presented in phase three. Each block had one $A\text{-}B^+$, $B\text{-}C^+$, $C\text{-}D^+$, and $D\text{-}E^+$ pair presented in a random order. Phase four consisted of 24 trials with pairs presented in a random order. Six trials of each pair were presented with no more than two identical trials occurring in a row.

Once phase four criterion was met, a probe session followed. The session began with eight baseline pair trials. Two of each pair were presented, and 7/8 correct was required for the rat to be given novel pairs. If 7/8 correct was not achieved, the remainder of the session consisted of phase four training. If the criterion of 7/8 was reached, the probe test was given. Probe testing included two presentations of $A\text{-}C^+$, $A\text{-}D^+$, $A\text{-}E^+$, $B\text{-}E^+$, $C\text{-}E^+$, $B\text{-}D^+$, $X\text{-}Y^+$, and one trial of each baseline pair. The $X\text{-}Y^+$ pair consisted of two novel scents, used to control for learning during the probe testing.

After completion of probe testing, two more days of training were given in phase five. Phase five included two trials of $A\text{-}C^+$, $A\text{-}D^+$, $A\text{-}E^+$, $B\text{-}E^+$, $C\text{-}E^+$, three trials of $B\text{-}D^+$ and $X\text{-}Y^+$, and two trials of each baseline pair. Following the two days of phase five, the second five-item list was trained. The second five-item list was trained in the same way the first list was trained.

Automated Procedure

Subjects

Four male Sprague-Dawley rats were trained for the automated procedure. They were kept at 85-90% of their free-feeding weight and given unlimited access to water. They were fed about 10-15 grams of Purina® Rat Chow after a minimum of 30 minutes after the completion of the testing session. The rats were kept on a 12 hour reverse light/dark cycle.

Apparatus

All housing and testing procedures were approved by the Institutional Animal Care and Use Committee. An operant chamber was used for training and testing (see

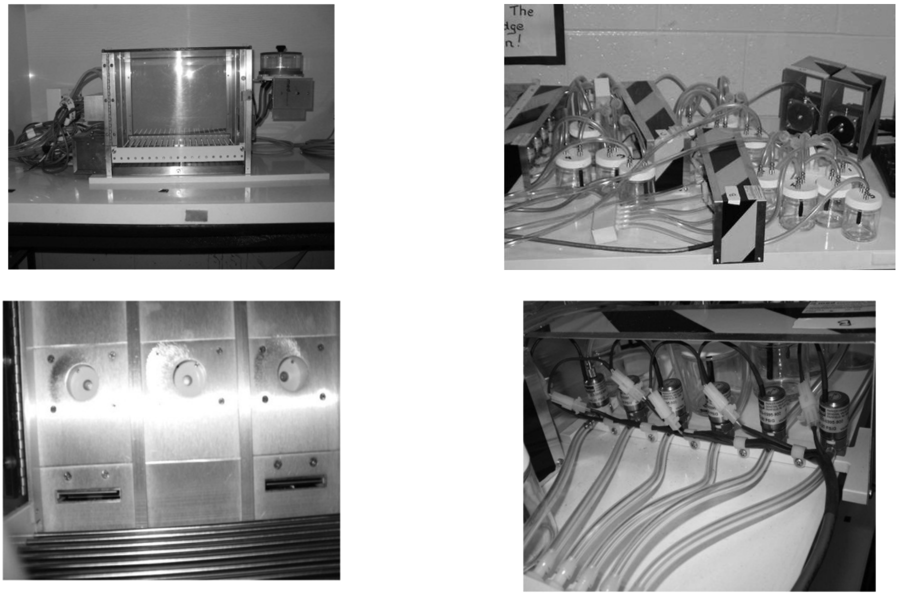

Figure 2. Automated apparatus. The operant chamber is shown in top left. The nose pokes on the left side of the chamber are shown in lower left. The top right image shows the olfactometers (where scented oil was stored). The lower right image shows the solenoids.

Figure 2A). The left side of the chamber had three nose pokes, with photo beam sensors that indicated when a response was made (see Figure 2B). Nose pokes were the response ports where scented air was pumped in. Only the left and right nose pokes were used in the transitive inference procedure. The right side of the chamber stored a pellet dispenser. Correct responses were reinforced by the presentation of a sucrose pellet. A house light was used to indicate the beginning and end of the session and to indicate a timeout after an incorrect response.

The stimuli used were scented oils from the Great American Spice Company. Peach, grape, bubblegum, strawberry, and banana were the scents used during baseline training and probe testing. Raspberry, vanilla, blueberry, and pineapple were used as unfamiliar scents during probe testing. Five ml of each scent was poured into each of three glass jars. One jar was attached to each olfactometer (see Figure 2C). One olfactometer was assigned to each nose poke. The jars were connected to the olfactometers by a lid that was connected to a pump. There was one pump per jar, and each pump was attached to a solenoid (see Figure 2D). Solenoids could be opened or closed, allowing for the corresponding scent to be pumped into the nose poke. A vacuum pump was used to constantly remove the scented air from the nose pokes to ensure that it did not spread into the chamber.

MedPC software was used to create the transitive inference programs. Four different programs were used in each phase. The programs were executed on a Dell desktop computer which was connected to the operant chamber.

Procedure

Magazine Training

One pellet was delivered and the hopper light turned on. The hopper was where the pellets were delivered, and the light was turned on to cue the rats that reinforcement had been delivered. The hopper light remained on for 45 seconds. After an intertrial interval of 15 seconds, another pellet was delivered and the hopper light turned on. This procedure was repeated 25 times, with a total of 25 pellets delivered. After the completion of the session, the pellets remaining in the hopper were counted. Criterion to move to any nose poke shaping was the consumption of all 25 pellets.

Any Nose Poke Shaping

Sessions ran for 25 minutes. Both the left and right nose pokes were activated, and a response to either nose poke resulted in reinforcement. One pellet was delivered and the hopper light remained on for five seconds. One disruption of the photo beam was counted as one response. Criterion to move to nose poke shaping was set at 25 responses in one session.

Nose Poke Shaping

Sessions consisted of 48 trials. For a trial, either the left or right nose poke was activated. A response to the activated nose poke resulted in reinforcement. Reinforcement consisted of the delivery of a pellet. The hopper light was turned on and remained on for five seconds. A response was defined as breaking the photo beam in the nose poke. For the first day of nose poke shaping, only one response was required to receive reinforcement (FR1). Each day, the fixed ratio was increased by one (e.g. FR2 on day two). If responding slowed or stopped, the fixed ratio was decreased. Criterion was set at 45 rewards on a fixed ratio of five responses.

Five-Item List Training

Rats were trained to form a hierarchy of five scents (A, B, C, D, and E) by presenting the scents in four pairs (A-B$^+$, B-C$^+$, C-D$^+$, and D-E$^+$). In each pair a response to one scent was always reinforced and response to the other scent never reinforced. Responses were counted when the rat broke a photo beam in the nose poke. A correct response resulted in the hopper light turning on for five seconds as a pellet was delivered. If an incorrect response was made, the house light immediately turned off and resulted in a five second timeout. After the five second timeout, the next session began. This non-correction procedure was utilized throughout all phases.

Each rat was trained with the five scents: peach (A), grape (B), bubblegum (C), strawberry (D), and banana (E) during the 48 daily trials. Phase one consisted of four blocks of 12 trials. Only the A-B$^+$ pair was presented in block one, followed by the B-C$^+$ pair in block two, the C-D$^+$ pair in block three, and the D-E$^+$ pair in block four. Correct responses were reinforced with one sugar pellet which was released into the hopper. The hopper light remained on for five seconds. Incorrect responses resulted in a five second timeout. Criterion to move to each phase was set at 10/12 (83%) correct on each type of pair for two consecutive days. In phase two, each pair was presented in blocks of six. A-B$^+$ was the first block, followed by B-C$^+$, C-D$^+$, and D-E$^+$ respectively. The blocks were then repeated so a total of 12 trials of each pair was completed. Phase three consisted of blocks of four trials. A-B$^+$ was the first block of, then B-C$^+$, C-D$^+$, and D-E$^+$. Once four trials of the D-E$^+$ pair were completed, A-B$^+$ pairs were presented again, followed by B-C$^+$, etc. This order was repeated three times for a total of 12 trials of each pair. In phase four, each pair was presented once, starting with A-B$^+$ and ending with D-E$^+$. This sequence was repeated twelve times. The final phase, phase five, consisted of blocks of four trials in which each pair was presented once but in a random order. For example, the first block may be B-C$^+$, A-B$^+$, C-D$^+$, and then D-E$^+$ and the next block could be C-D$^+$, D-E$^+$, B-C$^+$, and A-B$^+$. Each pair was presented twelve times for a total of 48 trials.

Probe Testing

Once criterion was met during phase five, probe tests were given. Probe tests consisted of the presentation of B-D$^+$ and X-Y$^+$. The pair X-Y$^+$ consisted of two novel scents which were used to control for learning the novel pairs. Twenty-four trials of both pairs were presented each session for three sessions.

Following B-D⁺ and X-Y⁺ tests, retraining on phase five was given. Once the rat met criterion, more probe tests were given. End anchors A and E were presented (A-E⁺) and two novel stimuli were also presented (W-Z⁺). Twenty-four trials of both pairs were presented each session for three sessions.

RESULTS

Manual Procedure

S16 met baseline training criterion in 90 sessions. Figure 3 shows a breakdown of the number of sessions per phase.

After meeting criterion for List One Training, S16 was given probe testing with every possible combination of stimuli. Table 1 shows the number of trials correct for each pair for S16 and two rats from Jordan's (2009) study, P39 and P23. All rats performed at above 90% correct for all transitive pairs. S16's total percent correct out of the 24 trials, including baseline trials but excluding the two trials with unfamiliar scents, was 100%. On the two presentations of B-D⁺, S16 scored 2/2 (100%); that is, he chose D over B both times. The number of correct trials with the unfamiliar scents (X-Y⁺) is also presented in Table 1. S16 performed better on the tests with transitive pairs compared to the tests with unfamiliar scents (1/2, 50% correct).

S16 also met criterion in all four phases during List Two training. The total number of training sessions was 59. Figure 4 shows a breakdown of the number of sessions per phase.

Table 2 shows the number of trials correct for each pair during probe testing for S16 and two rats from Jordan's (2009) study, P39 and P23. All rats performed at above 90% correct for all transitive pairs. S16's total percent correct, including baseline trials but excluding the two trials with unfamiliar scents, was 90.91%. On the two presentations of G-I⁺, S16 scored 2/2 (100%). The number of correct trials with the unfamiliar scents (W-Z⁺) is also presented in Table 2. S16 performed better on the tests with transitive pairs compared to the tests with unfamiliar scents (0/2, 0% correct).

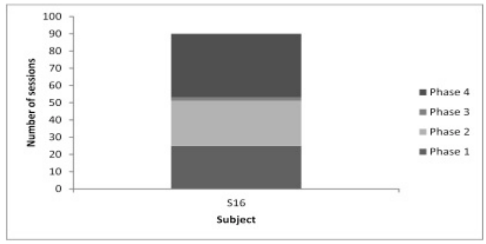

Figure 3. This graph shows the number of sessions per phase in the manual procedure for List One.

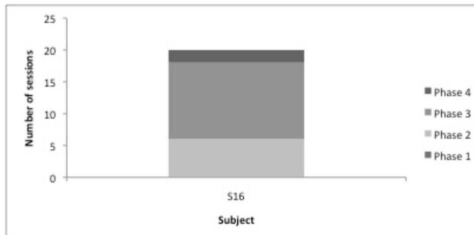

Figure 4. This graph shows the number of sessions per phase in the manual procedure for List Two.

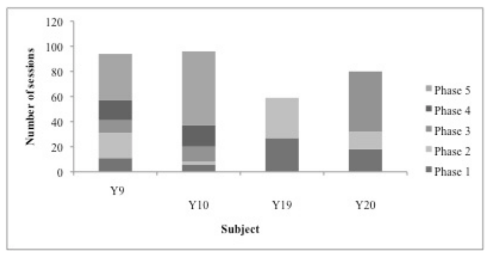

Figure 5. This graph shows the number of sessions per phase in the automated procedure for each subject, Y9, Y10, Y19, and Y20.

Automated Procedure

None of the rats trained with the automated procedure met criterion during baseline training. The mean number of sessions completed before training was terminated and subjects were either dropped from the study or given probe tests was 82.25. Figure 5 shows a breakdown of the number of sessions per phase each rat completed.

Y19 was dropped from the study using the automated procedure because of a failure to meet criterion in phase two.

Y9 was presented with the A-E^{+} and W-Z^{+} probe test in three days of probe testing. A binomial test was conducted for each day for percent correct on A-E^{+} trials and W-Z^{+} trials. However, the binomial test for choice of E on A-E^{+} trials was not significant on any of the three days (15/24, 15/24, 18/24, respectively, all $p>0.05$). The binomial test for choice of Z on W-Z^{+} trials on was not significant on days one and two (13/24 and 16/24, $p>0.05$). On day three, Y9's performance for choice of Z on W-Z^{+} trials was significant (17/24, $p<0.05$). Figure 6 shows the percent correct on the final day of baseline training and all three days of probe testing. Y9 shows little evidence of transitive inference; while performance on the A-E^{+} trial finally reached significance on day three, it was not striking. Overall, learning appeared to be similar to learning the relation between the unfamiliar scents, W and Z.

Y10 was also presented with the A-E^{+} and W-Z^{+} probe test with three days of probe testing. Again, the binomial test for choice of E on A-E^{+} trials was not significant on day one (16/24, $p>0.05$), but was on day two (17/24, $p<0.05$) and three (19/24, $p<0.01$). However, the binomial test for choice of Z on W-Z^{+} trials was significant on all three days (17/24, 17/24, and 22/24, all $p<0.05$). Figure 7 shows the percent correct on the final day of baseline training and all three days of probe testing. Y10's improvement in performance across the three days of probe testing provides some evidence for learning, however, no evidence for transitive inference. His performance was the same for A-E^{+} and W-Z^{+} trials.

Y10 was also presented with the B-D^{+}

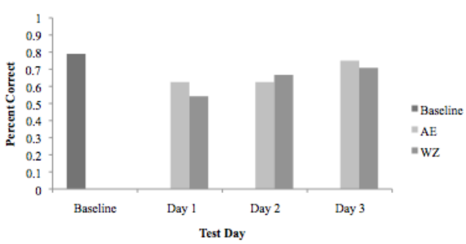

Figure 6. Y9's percent correct on the final day of baseline training and on each day of probe testing.

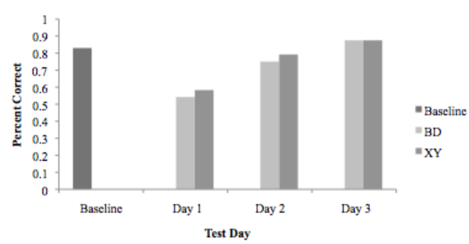

Figure 8. Y10's percent correct on the final day of baseline training and on each day of probe testing.

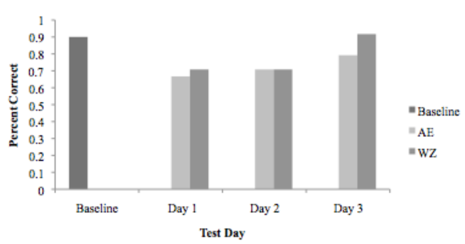

Figure 7. Y10's percent correct on the final day of baseline training and on each day of probe testing.

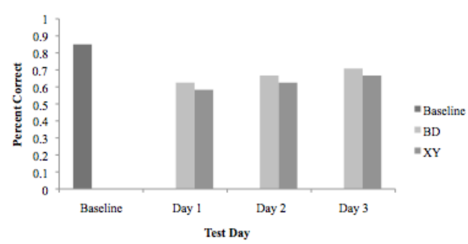

Figure 9. Y20's percent correct on the final day of baseline training and on each day of probe testing.

Table 1. Results from List One probe tests for S16 and two subjects from Jordan (2009), P39 and P23.

Subject	BD+	AD+	AC+	BE+	AE+	CE+	All novel transitive pairs	Baseline	XY (unfamiliar)	Total
P39	2/2	2/2	2/2	2/2	2/2	2/2	12/12*	3/4	2/2	90.91%
P23	2/2	2/2	2/2	2/2	2/2	1/2	11/12*	4/4	1/2	90.91%
S16	2/2	2/2	2/2	2/2	2/2	2/2	12/12*	4/4	1/2	100.00 %

Note. Total percent correct does not include X-Y+ data
*p< .01

Table 2. Results from List Two probe tests for S16 and two subjects from Jordan (2009), P39 and P23.

Subject	GI+	FJ+	FH+	FI+	HJ+	GJ+	All novel transitive pairs	Baseline	WZ (unfamiliar)	Total
P39	2/2	2/2	2/2	2/2	2/2	2/2	12/12*	3/4	1/2	90.91%
P23	2/2	2/2	2/2	2/2	2/2	2/2	12/12*	4/4	1/2	100%
S16	2/2	2/2	2/2	2/2	2/2	2/2	12/12*	3/4	0/2	90.91%

Note. Total percent correct does not include W-Z+ data.
*p< .01

and X-Y^+ probe test over three days. A binomial test was conducted for each day for percent correct on B-D^+ trials and X-Y^+ trials. The binomial test for choice of D on B-D^+ trials on day one was not significant (13/24, p>0.05), but was on days two (18/24, p<0.01) and three (21/24, p<0.01). The binomial test for choice of Y on X-Y^+ trials on day one was not significant (14/24, p>0.05), but was on day two (19/24, p<0.01) and three (21/24, p<0.01). Figure 8 shows the percent correct on the final day of baseline training and all three days of probe testing. Y10 shows evidence for learning over days. However, performance was identical for B-D^+ and X-Y^+ trials, so there is no evidence for transitive inference.

Y20 was presented with the B-D^+ and X-Y^+ probe test for three days. Figure 9 shows the percent correct on the final day of baseline training and all three days of probe testing. The binomial test for choice of D on B-D^+ trials was not significant on day one (13/24, p>0.05) or two (16/24, p>0.05), but was on day three (17/24, p<0.05). The binomial test for choice of Y on X-Y^+ trials was not significant on any of the three days (14/24, 15/24, 16/24, all p>0.05). Thus, Y20 showed no clear evidence of transitive inference or learning across the probe trials.

DISCUSSION

Manual Procedure

Overall, the results from the manual procedure show that S16 was able to make transitive inferences with both five-item lists. When presented with the novel pair B-D^+, S16 chose D over B both times. Also, when presented with the novel pair G-I^+, S16 chose I over G both times. The relations between novel pairs that emerged indicate that a hierarchy had been formed for both List One and List Two.

Automated Procedure

The results from the automated procedure were not very promising. Two of the four rats in the automated procedure never reached phase five, and of the two rats that reached phase five, only one was close to meeting criterion in phase five. Performance on the transitive inference probes was at chance levels.

Due to Y20's health, it was unclear how much longer he would be able to test, so he was given the B-D^+ probe test before he met training criterion. His poor performance during training and on the probe tests may be attributed to old age. Y9 was presented with A-E$^+$ probe tests because he reached phase five but could not meet criterion. Y9 had achieved 10/12 on each pair, just not on the same day. Y9 had been trained in all five phases, and we believed he would have been successful at the A-E$^+$ pair. The A-E $^+$ pair contains two end stimuli, and therefore, should be the easiest of all novel pairs to respond correctly to because the choice can be made based on reinforcement histories alone (Weaver et al., 1997). Y10 was the only rat to be tested on both the A-E$^+$ and B-D$^+$ pairs. He did not meet criterion in phase five. The decision to test Y10 on the B-D$^+$ pair was made because he had met criterion of 10/12 on each pair on several days, just never consecutively.

The rats' poor performances on novel probes in the automated procedure may be attributed to their failure to meet criterion during training. Other studies that involve abstract concepts, such as Schusterman and Kastak (1993), have shown that animals require extensive training before probe testing. Schusterman and Kastak demonstrated that a California sea lion was capable of forming equivalence relations, but only after extensive training. The California sea lion was extensively trained with multiple exemplars to criterion before novel tests of transitivity, symmetry, and equivalence were given. The success on the equivalence task may be due to the extensive training which prepared the sea lion for the probe tests. The rats in our automated procedure failed to fully meet the criterion for probe testing. Therefore, it may be argued that they did not receive the extensive training needed to form the five-item hierarchy.

Though the rats in the automated procedure did not reach criterion, they received much more training than the rats in the manual procedure. In the manual procedure, S16, was trained with 24 trials per session for 90 sessions. Rats in the automated procedure were trained with 48 trials per session for an average of 82.25 sessions. Despite the increased amount of training, the rats in the automated procedure did not meet criterion to receive probe tests, whereas S16 met criterion. This discrepancy between increased amount of training in the automated procedure and failure to meet criterion suggests that amount of training does not account for the automated procedure's failure. By examining some of the differences between the manual and automated procedure, possible errors and improvements for future training in the automated procedure can be discussed.

Comparing the Two Procedures

One difference between the manual and automated procedure was the spatial difference between response and reinforcement. Rats trained in the manual procedure received reinforcement immediately after making a response. Rats in the automated procedure were required to make a response on the left side of the chamber, but the reinforcement was delivered on the right side of the chamber. The spatial distance between where the response was made and reinforcement was received also increased the time between making a response and receiving reinforcement. In Otto and Eichenbaum's (1992) study using an automated apparatus to test delayed non-match-to-sample tasks, there was not a long time or spatial delay between response and reinforcement. Access to water was used as reinforcement, and the water port was located directly above the nose poke. After making a correct response, the rats' response was immediately reinforced. Otto and Eichenbaum's success in training rats on delayed non-match-to-sample tasks using an automated apparatus may be attributed to the minimal time between when rats made a response and when they were reinforced. Moving the pellet dispenser between the nose pokes in the automated procedure would decrease the spatial distance and time between when the rat makes a response and when reinforcement is delivered.

Using a photo beam may have also affected the rats' performances in automated apparatus. Rather than having to make an explicit response, such as pushing a lid in the manual procedure, rats were required to make a less salient response. The photo beam was not visible to the rats, nor could disrupting it be heard or felt. If the rats had difficulty learning what response was required, this may have lengthened the amount of training needed.

The manual procedure also trained rats with different scents than the automated procedure. The manual procedure used spices whereas the automated procedure used scented oils, and all of the oils used were fruity (e.g., strawberry, grape, and banana). Though to us the oils were distinguishable, we do not know how the scents smelled to the rats. If this procedure was repeated, it would be useful to use scents that are more different (e.g., pecan, butter, and champagne). In addition, it may be useful to standardize the types of scents (fruits vs. spices) across procedures. This was not done in the current study because of the limited variety offered by the manufacturers we used.

Ecological Significance

Another difference between the manual procedure and the automated procedure is the ecological significance of the required response. The importance of utilizing ecologically significant procedures was demonstrated in a study by Wright and Delius (1994). In this study, pigeons were trained on match and non-match-to-sample tasks using different gravel types as stimuli. The stimuli were chosen by observing pigeons' natural behavior of digging to find food. The pigeons learned the task at a much quicker rate than when traditional key-peck training methods are used. Traditional key-peck training with visual stimuli requires pigeons to peck at lights on a screen. The key-peck method is relatively "two-dimensional", not allowing extensive manipulation with the screen, but Wright and Delius (1994) found that by using three-dimensional stimuli that could be manipulated, the pigeons learned the task much quicker. These findings are similar to the findings in our transitive inference study. The manual apparatus that used three-dimensional scented lids was much more successful and faster at training rats to form a five-item hierarchy.

Delius (1992) trained pigeons to discriminate between spheres and non-spheres. The stimuli were three-dimensional objects made of various materials and textures. Acquisition was very fast compared to other procedures using two-dimensional stimuli. Once the pigeons were trained, they were presented with novel stimuli and were able to discriminate between novel spheres and novel non-spheres. Pictures of the objects were also tested, and though the pigeons did discriminate between spheres and non-spheres, performance was lower than performance with the three-dimensional objects. The results from Delius (1992) suggest that the pigeons learned to discriminate between three-dimensional object more quickly because the stimuli were more similar to stimuli found in the pigeons' natural environment. The pigeons' high performance and fast acquisition demonstrates the importance of using stimuli that are relevant to animals' environmental experiences. It is possible to explain our results in terms of three-dimensional and two-dimensional stimuli. In the manual procedure, the scents were on the three dimensional objects, Plexiglas lids. In the automated apparatus, the stimuli could be considered two-dimensional, like the pictures in Delius (1992). In order to make a response in the automated apparatus, the rats did not have to manipulate the stimuli. Delius found that acquisition and performance were higher with three-dimensional stimuli compared to two-dimensional stimuli. These results are similar to our findings that the manual procedure succeeded in training a five-item hierarchy, but the automated procedure required more training and was not successful in training a hierarchy.

If the automated procedure was more difficult for the rats to learn because it is not ecologically significant, rats in this procedure may require more extensive training. Training the rats with different programs may help them learn the hierarchy more quickly. For example, Davis (1992) trained rats to form a five-item hierarchy by training one pair at a time. In phase one, only the A-B pair was presented. In phase two, only the B-C pair was presented. Phase three trained rats on the A-C pair, and phase four only presented the C-D pair. Phase five presented the D-E pair, phase six trained the C-E pair, and phase seven trained the A-E pair. The rats received extensive training on each adjacent pair as well as nonadjacent pairs. This procedure may have been successful because the rats were given experience with nonadjacent pairs before the transitive inference test.

Similarly, before the test of equivalence in Schusterman and Kastak's (1993) study, the California sea lion was given multiple exemplars and had been trained and tested on tests or symmetry, transitivity, and equivalence. By first training the sea lion with symmetry, transitivity, and equivalence, the sea lion was familiar with these types of tests. Familiarizing our rats with the type of test that will be given on a probe day may be a useful addition to the automated procedure. Our automated procedure could be modified to train rats in a similar way as Lazareva and Wasserman (2006) and Davis (1992). Familiarizing our rats with the type of test that will be given on a probe day may be a useful addition to the automated procedure.

The wide range of species (e.g., macaque monkeys (Treichler & Van Tilburg, 1996), pigeons (von Ferson, Wynne, Delius, & Staddon, 1991; Lazareva & Wasserman, 2006), and rats (Davis, 1992; Dusek & Eichenbaum, 1997)) that have succeeded on transitive inference tasks may be related to abilities required for living in social groups. Animals living in social groups must be able to recognize others in their groups as well as monitor social rank and status. For example, male elephant seals fight for access to females and less than one-third of them actually mate each season (LeBouef, 1974). Body size is one of the factors that influences each males social rank. Central males are usually the largest and most experienced fighters. Peripheral males are restricted to the periphery of the social group, and outside males have little, if any access to females (Modig, 1996). Instead of fighting every male in the group, a newcomer can determine his place in the hierarchy based on his interactions with just a few individuals.

Male elephant seals may utilize physical characteristics of group members to form a hierarchy. However, hyenas are an example of a species that does not use physical dimensions when monitoring social rank. The social status of male hyenas is not based on physical characteristics, but is determined by the hyena's "place in line." Males essentially wait to rise in status rather than fight each other. Male hyenas that join a new group rarely use physical means to secure status. Rather, they are made aware of their status by a greeting ceremony. During the greeting ceremony, the new male is approached by another male of similar social status. By evaluating the male's relationship with all of the other members of the group, the new male is able to determine his social status (Slater, Rosenblatt, Snowdon, & Roper, 2002).

The need to monitor and evaluate social status based on relationship to other group members requires the formation of hierarchies. The formation of hierarchies in novel situations allows an animal to determine rank without fighting several group members. Rodents such as meadow voles, African striped mice, and rats have complex social interactions that result in the formation of dominance hierarchies (Spritzer, Meikle, & Solomon, 2004; Kinahan & Pillay, 2008; Calhoun, 1963). This capability is an adaptive strategy that requires the use of properties of transitive inference.

Conclusion

Thus, it is important to continue studying transitive inference in laboratory settings. While our results do not provide evidence that an automated apparatus is an effective tool for training rats in transitive inference tasks, Jordan's (2009) success in training rats in two five-item lists suggests that using a manual procedure is a preferred training method. However, using an automated apparatus has several advantages compared to using a manual procedure. For example, objectivity and efficiency are increased. Therefore, improvements to the automated apparatus should be made before it is determined that the automated procedure should not be used in training rats in transitive inference tasks.

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