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Acquisition and Limited Transfer of Numerical Discrimination of Object Stimuli in

Rats

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Abstract

A series of experiments explored rats' ability to learn abstract ordinal position of object stimuli to investigate their numerical competence. Three of four Long-Evans rats, trained to respond to the third of six objects in a line, reliably learned this task in three different trials with three different stimulus objects. As the objects' spatial location was changed trial-by-trial, spatial position of stimuli could not serve as an effective discriminative cue. In the first transfer test, trials with three novel objects were used as probe tests to the original training. In the second test, rats were trained with all six objects, and then given three novel test stimuli. During the transfer test period, rats maintained good performance with training stimuli, whereas most responses to probe tests were at chance level, showing limited transfer of counting behavior to novel stimuli. Results are discussed in terms of stimulus-specific learning and domainrestricted concept learning.

Keywords: rats, counting, numerical competence, stimulus-specific learning, domain-restricted learning

A numerical concept allows processing of the numerical aspects of aggregations that differ in physical features. For example, sets of pencils, apples, and cars differ in various dimensions, but the numerical aspect of these sets of objects can be processed by a common cardinal number, such as "3". By means of an abstract numerical concept, we can apply common calculation rules to different stimuli. Such cognitive manipulation of numerical concepts may have some benefit for non-human animals. For example, when an animal tries to identify its nest, if it can recognize that it is in the third tree from a certain vantage point, it might be able to identify the correct spatial location of the nest despite seasonal changes in specific features of trees.

A common theoretical framework has been proposed to elucidate basic numerical competence in human infants and non-human animals (see Brannon & Roitman, 2003; Spelke, 2011, for reviews). The abstract concept of number consists of two major components, cardinality and ordinality (Gelman & Gallistel, 1978; Brannon & Roitman, 2003). Cardinality refers to absolute numerosities, the abstract number of stimuli in an aggregation, while ordinality indicates an ordinal judgment among those cardinal numbers, e.g., "3 is greater than 2 and less than 4".

Many researchers have demonstrated that various species can learn nonverbal numerical tags. For example, chimpanzees can respond correctly to the number of visual stimuli on a screen using Arabic numerals (Matsuzawa, 1985) or collect an appropriate number of dots on a computer monitor, corresponding to a specified Arabic numeral, using a joystick (Beran & Rumbaugh, 2001). Boysen and Berntson (1989) trained a chimpanzee to give the sum of oranges hidden in different places using Arabic numerals. Rhesus monkeys (Brannon & Terrance, 1998, 2000) can judge the ordinal relationship of a number of different figures on a computer monitor. Pigeons can learn a symbolic matching-to-sample task in which they have to respond to symbols representing numbers (Xia, Emmerton, Siemann, & Delius, 2001; Xia, Siemann, & Delius, 2000), while an African Grey parrot could give a vocal answer to the number of objects or the sum of two cardinal numbers (Pepperberg, 1994, 2012). Infant chicks are capable of choosing relatively larger object sets over smaller ones or finding a target hole according to a specific ordinal position in a line, suggesting that numerical ability for small numerosities is innate (Rugani, Fontanari, Simoni, Regolin, & Vallortigara, 2009; Rugani, Kelly, Szelest, Regolin, & Vallortigara, 2010; Rugani, Regolin, & Vallortigara, 2007; Rugani, Vallortigara, Vallini, & Regolin, 2011).

While primates and avians appear to demonstrate some understanding of cardinality and ordinality, studies have also been conducted on numerical competence in rodents. Apparently rats can discriminate the number of reinforced runs in a runway (Capaldi & Miller, 1988; Burns, Goettl, & Burt, 1995), the number of touches to their body (Davis, MacKenzie, & Morrison, 1989), the number of auditory tones (Davis & Albert, 1986; Breukelaar & Dalrymple-Alford, 1998), the number of electrical foot shocks (Davis & Memmott, 1983), and the number of lined tunnels in an open field (Davis & Bradford, 1986; Suzuki & Kobayashi, 2000). These studies controlled some physical aspects of the stimuli, such as total duration of a tone or spatial positions of tunnels, to prevent them from being used as effective discriminative cues. Given that the transfer of counting behavior to novel stimuli has not yet been examined in rodents, abstractness of numerical concepts in rodents has not been elucidated. The exception was a study by Davis & Albert (1987), which tested the transfer of numerical discrimination of auditory stimuli to visual stimuli in rats, but there was no apparent transfer of learning.

The goal of the present study is to examine whether rats can learn a specific abstract ordinal position of a stimulus by examining acquisition and transfer of numerical discrimination of object stimuli. Figure 1 shows a schema of the apparatus and stimulus objects used in the present study. Figure 2 demonstrates the placement of objects in different trials. In any given trial, four, five, or six identical objects were placed in front of goal boxes 1 through 10. Rats were trained to choose the third object from the left in the line of goal boxes. Assignment of objects to goal boxes was changed trial-by-trial to prevent a specific spatial location from serving as a discriminative cue.

If rats could learn to respond to the third object and transfer this discrimination to novel objects in this task, it would evince acquisition of the concept of abstract ordinal position, without regard to specific physical features of objects. In monkey and pigeon studies of the abstract same/different relational concept, animals shifted their learning strategy from stimulus-specific learning to abstract relational learning as the variety of training stimuli increased (e.g., Katz & Wright, 2006). Therefore, we repeated training and testing phases with an increasing variety of training stimuli in the expectation that a larger number of training exemplars would facilitate learning of abstract ordinal position.

Table 1 outlines the experimental training and test phases. First, rats were trained with object A (Training phases 1-4), and then objects B and C were added (Training phase 5). After completion of training with objects A, B, and C, a transfer test with novel object D was conducted (Test phase 1). Then training and testing were repeated with an increasing number of training objects (Training phases 6-9 and Test phases 2-5). On all the test trials, except the last with objects X, Y, and Z, we reinforced not only the third object but also the second and fourth objects. This procedure was adopted as a modification of non-differential reinforcement to allow researchers to eliminate possible effects of additional learning to the test stimuli during test periods (e.g., Castro, Lazareva, Vecera, & Wasserman, 2010). We restricted reinforcement to only the second, third, and fourth objects because our preliminary investigation found that non-differential reinforcement to all test stimuli disrupted rats' baseline performance, precluding any examination of transfer performance.

Transfer tests with object F were repeated twice (Test phases 3-4) in order to evaluate the effect of familiarity of test stimuli. D'Amato, Salmon, & Colombo (1985) suggest that novelty of test stimulus interferes with discriminative responses by provoking exploring behavior toward the novel stimuli. If increasing familiarity of test stimuli contributed to a decrease of the interference effect on test performance, it would be expected that test performance with object F should improve with repetition of testing.

Method

Subjects

The subjects were four experimentally naïve, male Long-Evans rats, approximately 80 days old at the start of the experiment, whose initial ad-lib body weights were 247 g, 295 g, 257 g, and 252 g for Rats 1-4, respectively. Rats were given 14 g of food daily except for experimental rewards. These rats were acquired from Kiwa Laboratory Animals Co., Ltd. and were cared for and used according to guidelines approved by Kanazawa University Animal Experimentation Regulations.

Apparatus

Top of Figure 1 shows a schematic representation of the apparatus. Ten goal boxes, 30 cm long, 10 cm wide, and 40 cm high, were lined up next to each other in the apparatus. Each goal box had a one-way swing door at its entrance and a small oneway door 25 cm beyond the entrance. A rectangular food cup was placed behind the small one-way door, and three grains (30 mg) of sweetened puffed rice were placed in the cup as a food reward. One side of the food cup was 5 cm high and the remaining three sides were 1 cm high. Rats could open the inside door and get the food reward only when they had chosen the correct goal box. The inside doors of incorrect boxes were blocked by positioning the food cup so that the high side of the cup obstructed the inside door and rats could not reach the reward. The apparatus was painted flat gray, and the one-way doors were made of gray PVC board.

Bottom of Figure 1 shows a photograph of the 12 types of object stimuli used in the experiment. Objects used in the initial training and transfer test were brown glass bottles, wine glasses, green clothespins, metallic containers, white hemispherical capsules, and ceramic dolls. Three of the six object stimuli were used as training stimuli (A, B, and C), while the rest were used as test stimuli (D, E, and F) in Test phases 1-4. The assignment of object stimuli was counterbalanced among subjects. In Test phase 5, white translucent plastic bottles, light blue plastic cups, red aluminum cans, PVC joint pipes, alarm clocks, and white conical cups were used for training, non-differential-reinforcement testing, and differential-reinforcement testing. The assignment of objects was counterbalanced among subjects. Each stimulus object was fixed on a gray PVC board, 8 by 8 cm and 1 mm thick.

Procedure

Pre-training: During the first 11 days, rats were handled for three minutes and ten grains of sweetened puffed rice were placed in their home cage from the 7th to the 11th day to familiarize them with the experimental rewards. Exploration of the apparatus was allowed on days 12 and 13, when rats could investigate it freely for 20 minutes. All doors were open and rats could eat the sweetened puffed rice scattered over the apparatus floors and food cups. Following the same procedure, 10 minute free exploration sessions were allowed twice a day on days 14 and 15. From day 16 to 35, shaping sessions trained the rats to open the one-way doors. A rat was put in the start box and then the guillotine door was opened about three seconds later. Nine of the 10 doors were blocked and rats could only enter the single open goal box. Rats were eventually trained to enter a completely closed door. This training was repeated for 20 trials per day.

Experimental training: Training phase 1 began on day 36, first training rats with stimulus A, with object stimuli counterbalanced across subjects. Object stimuli were arranged in random order in front of the 10 goal boxes, and the rats were trained to choose the third object, counting from left to right, from the array of objects. The total number of object stimuli varied randomly from four to six objects every three trials. To control olfactory cues, food rewards were placed in all the food cups inside the goal boxes, but the small one-way door could only be opened in the correct box. In the sixobject condition, possible positions for the correct goal box were the third, fourth, fifth, sixth or seventh in the row of 10 boxes. For the four- and the five-object conditions, we maintained the same possible positions for the correct goal box as in the six-object condition, thereby excluding the eighth, ninth, and tenth boxes as correct goal boxes. The frequency of each of the five goal boxes as the correct box was made equal and counterbalanced over five trials, and 20 trials were conducted in their daily session. Rats were trained by a correction method in which they were returned to the start box and allowed to choose an object in the same distribution when they made an incorrect response. For the first 30 sessions (Training phase 1), the number of repetitions per trial was not restricted, and for next 12 sessions (Training phase 2), repetitions were restricted to two (Table 1). Then, the rats were trained under the condition of only a single repetition allowed until they achieved the learning criterion of 70% correct over two consecutive sessions (Training phase 3). If the rats attained the criterion level, they were trained using a non-correction method with no repetitions allowed for incorrect responses (Training phase 4). After the rats attained the learning criterion of 70% correct using the original object stimulus, the variety of training object stimuli was increased from one to three by adding objects B and C (Training phase 5). Only one kind of object stimulus was used in a trial. Trials with these three objects were given in

random order in each three-trial block and there were 6 2/3 blocks in each 20-trial session. The learning criterion was set at 75% correct over two consecutive sessions.

Test phase 1 began the day after completion of acquisition training. Test trials with a new stimulus D were inserted in training trials using stimulus A, B, or C. On the test trials, responses to the second, third, and fourth object stimulus from the left in the line were reinforced non-differentially. The reasons for reinforcing these three goal boxes are as follows: by reinforcing the second, third, and fourth objects, we could evaluate two possible non-transfer effects, i.e., perception of the blockage of the inside door and training effects during the test period. It should have been impossible for rats to visually perceive whether the inside door was blocked or not because it was blocked by repositioning the food cup behind it. But if rats could somehow perceive that the inside door was blocked, but not the numerical cue of the object stimulus, they should respond to the second, third, and fourth objects equally. Similarly, if rats learned to respond to novel test stimuli based on reinforcement contingency, rats also should respond to these three objects equally because a response to all three stimuli was reinforced equally. Conversely, if a rat responded to the third object on a test trial rather than the second or forth despite non-differential reinforcement, it could be attributed to a transfer effect from acquisition training. Twenty training trials were conducted per session, with three test trials inserted into the sixth, 12th, and 18th trials, and Test phase 1 was conducted over a total of 10 sessions.

After the transfer test (Test phase 1), rats were trained with four kinds of stimuli, A, B, C, and D, as reacquisition training (Training phase 6). When they achieved the learning criterion of 75% correct responses over two consecutive sessions of in Training phase 6, a transfer test with stimulus E was conducted (Test phase 2). After the rats achieved the learning criterion of 75% correct of reacquisition with A, B, C, D, and E (Training phase 7), a transfer test with F was conducted (Test phase 3). Then the rats were retrained with stimulus A, B, C, D, and E (Training phase 8), and when they achieved the learning criterion of 75% correct, a second transfer test with stimulus F was conducted (Test phase 4). The procedure for Transfer tests 2-4 was identical to that of Transfer test 1, except for training and test objects.

After completion of Test phase 4, rats were trained with six different kinds of objects, A, B, C, D, E, and F (Training phase 9). When they had achieved the learning criterion of 75% correct response over two consecutive sessions, a test, with six novel stimuli G, H, I, X, Y, and Z, was conducted (Test phase 5). Three of the six novel objects, G, H, and I, were used as the "non-differential-reinforcement" test stimuli as in previous tests, and responses to the second, third, and fourth stimuli were reinforced non-differentially as was done in Test phases 1-4. Objects X, Y, and Z were used as "differential-reinforcement test stimuli", presented in same manner as the test stimuli, but reinforced only when rats responded to the third object, just as in the training stimuli. Differential-reinforcement test stimuli were introduced in order to enhance the response to the third object of the novel test stimuli and to evaluate possible effects of reinforcement manner on test results. Either a differential-reinforcement test trial or a non-differential-reinforcement test trial was inserted every three training trials. Each trial with novel objects G, H, I, X, Y, or Z was conducted once per session. Thus, 24 trials, including 18 training trials, three differential-reinforcement test trials, and three non-differential-reinforcement test trials, were conducted in a session. Rats were given 15 sessions for Test phase 5.

Results

Figure 3 shows the percentage of correct responses during Training phases 1-5. The solid vertical line in each plot represents the point at which rats started training with three objects (Training phase 5). Dotted lines represent the chance level. There could be different levels of chance during the acquisition training phase. One, set at 20%, is a mean of 1/4, 1/5, 1/6, which is the expected value when rats respond at random to four, five, or six presented objects. The second is also set at 20%, representing rats that respond randomly to five possible goal boxes, when the third to the seventh box could be correct. The third chance level is set at 35.01% of correct responses, when rats combine these conditions, that is, rats respond randomly to one out of 4-6 objects in front of one out of five possible goal boxes. Thus, we assume that 35.01% would be the most conservative chance level to evaluate a rat's performance. A score of 55% correct responses (11/20) was significant for performance in a 20-trial session using the 35.01% chance level (p = .02, binomial test, one-sided test). The learning criterion of over 75% in two consecutive sessions was attained by Rat 1 in 111 sessions, by Rat 2 in 89 sessions, and by Rat 3 in 77 sessions. Although Rat 4 showed statistically significant performance on 99 sessions out of 142 sessions with three kinds of objects, he could not attain the learning criterion within a total of 234 acquisition sessions.

To analyze the effect of the total number of object stimuli on a trial, each rat's performance for each condition is shown in Figure 4. There was no clear difference in correct performance by the total number of objects, and the main effects of Number of objects (F(2, 4) = 0.23) and Training phase (F(3, 6) = 1.22) and interaction of Number of objects * Training phase (F(6, 12) = 0.50) were not significant in a Number of objects (3) * Training phase (4) * Subjects analysis of variance.

Figure 5 shows the results of transfer tests with novel stimuli D, E, and F (Test phases 1-4). We reinforced responses to the second, third, and fourth object stimuli in the row to control for learning effects during test trials. Thus, the chance level was 1/3 = 33.33% to learn the correct response to the third object by reinforcement on test trials. However, because the chance level of 35.01% was more conservative, we used 35.01% to

evaluate rats' performance during the transfer test. Although generally they showed chance performance with most of the test stimuli, some rats' performance was significant with some test stimuli, namely the first (F) and second (F₂) test with object F by Rat 2 and the test with object D by Rat 3 (p < .05, binomial test, one-sided test). For example, on the first trial, Rat 2 responded correctly on the first F test (Test phase 3, Trial 1) but not the second (Test phase 4, Trial 1), and Rat 3 responded correctly for the test with object D (Test phase 1, Trial 1).

To evaluate the stability of test performance, rats' test performance was analyzed in five blocks of two sessions. Mean percentage of correct responses were 36.11%, 36.11%, 29.17%, 45.83% and 36.11% for the five blocks respectively and there was no systematic increase during the course of the repeated test sessions. The main effect of Block (F(4, 8) = 1.42) and Training phase (F(3, 6) = 0.24) and interaction of Block* Training phase (F(12, 24) = 1.17) were not significant in a Block (5) * Training phase (4) * Subjects analysis of variance. For the tests in which Rat 2 (F and F₂ tests) and Rat 3 (D test) showed significant performance, mean percentage of correct responses were 62.50%, 62.50%, 37.50%, 50.00% and 58.33% for the five test blocks respectively with no systematic increase in performance during test sessions observed. Hence, transfer performance seemed stable and did not reflect possible additional learning to test stimuli through reinforced experiences during the test period.

Rats 1, 2, and 3 attained acquisition training in 17, 15, and 2 sessions for training with objects A, B, C, and D prior to the test with object E, in 5, 6, and 18 sessions for training with objects A, B, C, D, and E prior to the test with object F, and in 3, 2, and 3 sessions for retraining with objects A, B, C, D, and E prior to the second test with object F, respectively. In contrast to original training with objects A, B, and C that required 77-111 sessions for completion, reacquisition with added object stimuli was attained quickly in a much smaller number of sessions. Figure 6 shows the distribution of responses to each goal box (left panel) and to each ordinal position of object stimulus (right panel) for training and test trials during Test phases 1-4. Almost all responses were to possibly correct goals (Boxes 3-7), and response rates to the goal boxes were generally the same for both training and test trials. For ordinal position, rats' responses concentrated on the third object and showed a sharp generalization gradient on training trials. By contrast, although rats' responded frequently to the third object even on test trials, they also responded to the second and fourth objects to a considerable extent and showed a moderate generalization gradient curve. Importantly, Rat 2 and Rat 3 also responded more frequently to goal boxes with no object stimuli on test trials than on training ones. Distribution of response rates to each goal box did not differ statistically for Rats 1-3: $\chi^2(4) = 3.242$, ns, $\chi^2(4) = 2.008$, ns, $\chi^2(4) = 1.815$, ns, respectively. Response rates to the second object were significantly lower on training trials than on test trials for Rat 1 ($\chi^2(1) = 40.289$, p < .05) and Rat 3 ($\chi^2(1) = 24.052$, p < .05), but such differences was not significant for Rat 2 ($\chi^2(1) = 1.559$, *ns*). For all rats, response rate to the third object was significantly higher on training trials than on test trials ($\chi^2(1)$ = 19.435, p < .05, $\chi^2(1) = 28.679$, p < .05, $\chi^2(1) = 34.389$, p < .05, for Rats 1-3 respectively). Response rate to the fourth object was significantly lower on training trials than on test trials for all rats ($\chi^2(1) = 7.269$, p < .05, $\chi^2(1) = 11.889$, p < .05, $\chi^2(1)=32.051$, p < .05, for Rats 1-3 respectively). Response rates to the goal boxes with no object stimulus was significantly higher on test trials than on training trials for Rat 2 ($\chi^2(1) = 13.968$, p < .05) and Rat 3 ($\chi^2(1) = 14.715$, p < .05), but not for Rat 1 ($\chi^2(1) = 14.715$) 0.452, *ns*).

Rat 2 and Rat 3 attained the learning criterion of the reacquisition training with A, B, C, D, E, and F (Training phase 9) in 12 and 16 sessions respectively. Because Rat 1 could not attain the learning criterion of Training phase 9 within 53 sessions, its training was stopped. Figure 7 shows the percentage of correct responses for training

stimuli, A, B, C, D, E, and F, for non-differential-reinforcement test stimuli, G, H, and I, and for differential- reinforcement test stimuli X, Y, and Z in Test phase 5. Although Rats 2 and 3 maintained relatively good performance with the training stimuli (p < .05, binomial test, one-sided test), they showed chance performance not only for the nondifferential-reinforcement test stimuli, but also for the differential-reinforcement test stimuli, for which responses to third object were reinforced selectively. Rats 2 and 3 responded to the third stimulus of the non-differential-reinforcement test stimuli (Rat 2; $\chi^2(1)=12.544$, p < .05, Rat 3; $\chi^2(1)=18.682$, p < .05) and the differential-reinforcement test stimuli (Rat 2; $\chi^2(1)=6.364$, p < .05, Rat 3; $\chi^2(1)=13.912$, p < .05) significantly less than that of the training stimulus. They also responded more frequently to goal boxes with no object for the non-differential-reinforcement test stimuli (Rat 2; $\chi^2(1)=28.600$, p < .05, Rat 3; $\chi^2(1)=7.076$, p < .05) and the differential-reinforcement test stimuli (Rat 2; $\chi^2(1)=11.034$, p < .05, Rat 3; $\chi^2(1)=29.985$, p < .05) than the training stimuli. Additionally, Rat 3 responded more frequently to the first ($\chi^2(1)=7.964$, p < .05) and second stimuli ($\chi^2(1)=17.389$, p < .05) of the test stimuli than that of the training stimuli.

Discussion

This study examined object stimuli counting behavior in rats. If they could learn the abstract ordinal position of the third stimulus in an object row, one would expect that rats could learn this task regardless of stimulus type and be able to transfer this numerical discrimination to novel stimuli. Three out of four rats attained the learning criterion during acquisition training with three different kinds of stimuli, and two of them also learned the task with six different kinds of stimuli. Since food rewards were placed in all goal boxes throughout the experiment, the possibility that rats used an olfactory cue could be excluded. Given that the total number of object stimuli was changed for each trial, alternating among four, five or six objects, it would be impossible to identify the correct stimulus in terms of its relative position in a row of objects. For example, if the total number of objects were six, the target third object would be placed just to the left of the halfway mark of objects in the row. However, the correct stimulus was placed at a different relative position when the total number of objects. Therefore the relative position of the correct stimulus in the row of objects could not serve as an effective discriminative cue. The possibility of counting from the far end of the row was also excluded because the ordinal position of the target object from the right end varied, depending on the total number of stimuli. Therefore, the performance of rats during acquisition training cannot be explained in terms of an olfactory cue, a relative position cue, or by counting from the right end.

As mentioned in the Results section, the most conservative chance level was 35.01%, and the performance of three out of four rats was significantly better than chance level. Although Rat 4 could not attain the learning criterion of 75% correct, its performance was statistically significant in 99 out of 142 training sessions using three kinds of object stimuli. These results suggest that rats can successfully perform object-counting tasks using this experimental set-up.

As described in the Method section, we adopted a food deprivation method of providing a constant amount of food per day, without regard to body weight. Body weight of Rat 4, and thus the ratio of daily food to body weight, was almost equivalent to that of the other rats, and Rat 4 was as active as the others per informal observations. Given these facts, lower motivation should not be a cause for failure of acquisition in Rat 4. The apparatus and task in the present study were modeled after Davis and Bradford (1986), in which rats were trained to respond to a specific tunnel ordinal position in a row of tunnels. Both variants of the apparatus allow rats to encounter object stimuli in multiple ways. For example, rats might encounter each object serially from first to last, starting from the left, or they could go to a distant object first and then move back and forth along the object row. In the latter case, serial enumeration of object stimuli might be more difficult. Unfortunately, their exploratory routes were not recorded, so we cannot speculate on whether individual differences in exploration resulted in poor performance for Rat 4. In a future study, it would be worth examining whether a modification of the apparatus to ensure that rats encountered the stimulus objects serially might affect rats' performance.

As mentioned above, numerical competence of primates and avians has been well documented with clear transfer of counting behavior to novel stimuli. When we examined transfer of counting behavior to novel test objects of different sizes, shapes, and colors, compared with training objects, we expected that rats would show reliable transfer of discrimination to novel test stimuli if they could learn numerical concepts at an abstract level like primates and avians. Although some rats learned the acquisition task with three or six kinds of object stimuli at more than 75% correct and maintained this good performance with the training stimuli during transfer tests, a clear transfer of numerical discrimination to novel test stimuli was not observed.

There might be several explanations for this lack of discrimination learning transfer in the present study. First, rats might learn to respond not only to the third test object, but also second and fourth, because the second and fourth objects were also reinforced on test trials. However, chance performance was observed, not only for the normal test stimuli, but also for the differential-reinforcement test stimuli that were novel but reinforced only for the third object as training stimuli, in Test phase 5 with

differential-reinforcement test stimuli X, Y, and Z (Figure 7). If non-differential reinforcement were a significant cause of chance test performance, better performance for differential-reinforcement test stimuli than for non-differential-reinforcement test stimuli should have been observed. Since rats performed slightly better on differentialreinforcement test trials than on non-differential-reinforcement test trials, the reinforcement schedule for novel stimuli might have some effect on test performance. However, performance was clearly poorer on differential-reinforcement test trials than on training trials and was not significantly better than chance for the differentialreinforcement test stimuli. Therefore, we cannot attribute poor test performance to non-differential-reinforcement of test stimuli. This result also excludes the possibility that rats responded simply to a blockage cue in the inside door. If rats had learned to perceive and respond to the unblocked inside door, then rats should have responded to the differential-reinforcement test stimuli equally as well as to the training stimuli because the inside door was unblocked only for the goal box of the third object. Therefore, significant performance with training stimuli and chance performance with differential-reinforcement test stimuli, shown in Figure 7, do not support the hypothesis that rats responded to an unblocked inner door cue.

Second, it is known that exploration of novel stimuli in monkeys interferes with test performance (D'Amato, Salmon, & Colombo, 1985). In our study, test performance might be disturbed by an exploratory tendency, given the novel stimuli presented in the test trials. Oden, Thompson, & Premack (1988) examined matching concept learning in infant chimpanzees, using object stimuli that were familiar to them, and successfully showed clear transfer to novel stimuli after acquisition training with a relatively small number of training stimuli. If we had used object stimuli that were familiar to the rats, a possible exploratory tendency might have been suppressed and might not have interfered with counting behavior on test trials. However, stimulus F was used in a second test (Test phase 4) after reacquisition training during Training phase 8. Familiarity with stimulus F should have increased in the second test compared with the first. Increased familiarity in the second test should have led to improved test performance by reducing any exploratory tendency, but no such improvement was observed. Thus, it might be difficult to explain the absence of a clear transfer to novel stimuli in terms of the interference of exploratory behavior on novel test stimuli. However, as shown in Figures 6 and 7, Rats 2 and 3 responded to goal boxes with no object stimulus more frequently on test trials than on training trials (Figure 6). This result might reflect some type of neophobia, an avoidance tendency toward novel test stimuli. Given these results, it is worth reexamining numerical discrimination learning and its transfer using familiar test objects.

A third possibility is that rats did not learn the abstract ordinal position of the target stimulus: rather, they acquired some kind of stimulus-specific learning for each of the training stimuli. Wilson, Mackintosh, & Boakes (1985) reported that pigeons could learn stimulus-specific conditional discrimination but not abstract same/different rules in a matching-to-sample task. Katz & Wright (2006) had similar results for a same/different discrimination task of two stimuli, when a small number of training stimuli were used, but pigeons possibly applied abstract same/different rules for novel stimuli when trained with large stimuli sets. In our study, rats might learn some stimulus-specific cue for each of the training stimuli rather than an abstract numerical cue. For example, rats might be able to identify the target third object by learning the rough quantitative amount of the first and second objects for each stimulus type and then choosing the next object beyond this specific aggregate cue. Although such learning would require rats to learn several different stimulus-specific cues for different object types, it would not require abstract numerical learning. Given that these cues are stimulus specific, rats would not be able to apply them to novel test stimuli. Studies of same/different relational concepts in primates and avians have shown that learning strategies shift from stimulus-specific conditional learning to abstract concept learning as the variety of training stimuli increases (Wright & Katz, 2006). The more training stimuli used, the greater the memory load required for stimulus-specific learning. But if animals could learn an abstract cue that could be applied generally to various stimuli, the memory load required for learning would be reduced. Thus, an increased number of training stimuli may facilitate learning an abstract numerical cue in an object counting task by rats.

A fourth possibility is that rats may learn abstract ordinal position of the training stimuli by using domain-restricted learning. Recently, in same/different relational concept studies with pigeons and monkeys, it has been posited that they may learn same/different relational learning that is limited to particular domains when they are trained with a small number of stimuli (Elmore, Wright, Rivera, & Katz, 2009; Wright & Katz, 2009; Wright & Lickteig, 2010). Stimulus-specific learning is strictly limited to specific features or configurations of training stimuli, whereas restricted-domain concept learning can apply to a variety of stimuli that are derived from training stimuli. Stimulus-specific learning predicts no transfer of learning to unfamiliar configurations or distorted versions of familiar training stimuli, whereas restricted-domain concept learning predicts transfer of learning to those stimuli but not to a set that consists of novel stimuli. For example, Wright & Katz (2009) trained pigeons and monkeys on same/different discrimination using two pictures and tested them with a novel combination or an inverted version of the training stimuli, in addition to completely novel stimuli. Pigeons and monkeys showed 70-90% correct performance, almost equal to that of the training stimuli, for novel combinations or inverted versions of the training stimuli, but they showed chance performance for novel stimuli.

Restricted-domain concept learning may explain the present findings of acquisition of the counting task with various stimuli but no transfer to novel stimuli. Further investigation with detailed testing that manipulates the combination, inversion, or distortion of training stimuli is needed to clearly distinguish stimulus-specific learning from restricted-domain numerical learning in rats. The present study demonstrates that rats can learn a numerical discrimination task with object stimuli varying in size, shape, and color. However, whether rats can learn the abstract ordinal position of a target stimulus is still unclear. Acquisition training with greater stimulus variation and modified testing will be needed to clarify these questions of numerical competence in rats.

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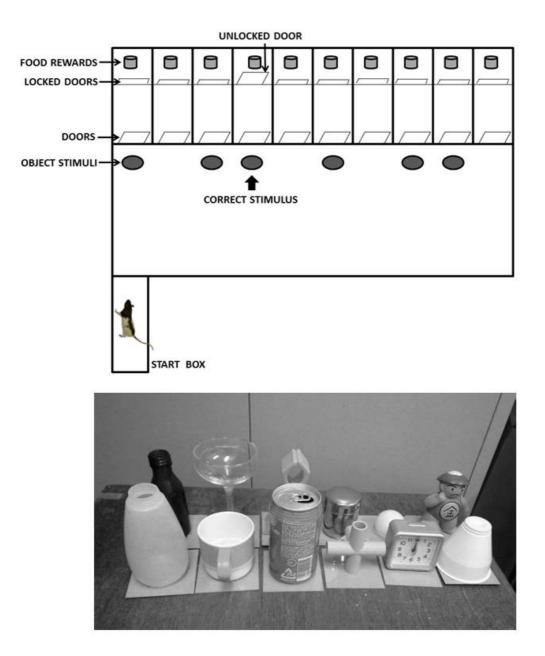
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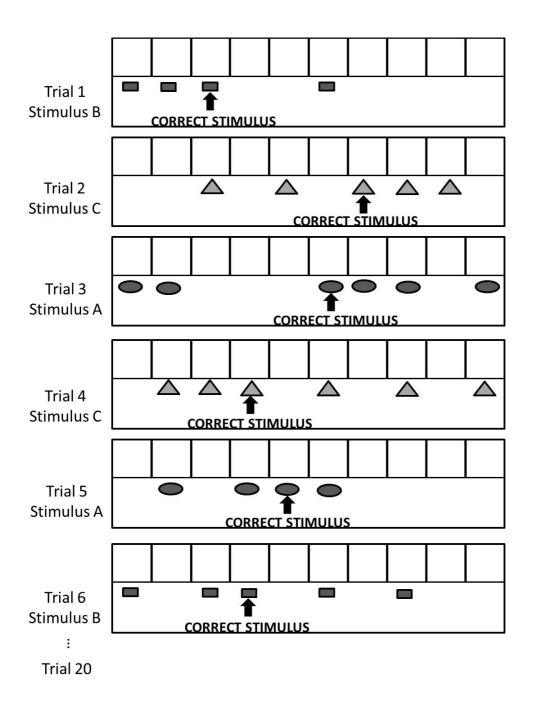
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Caption

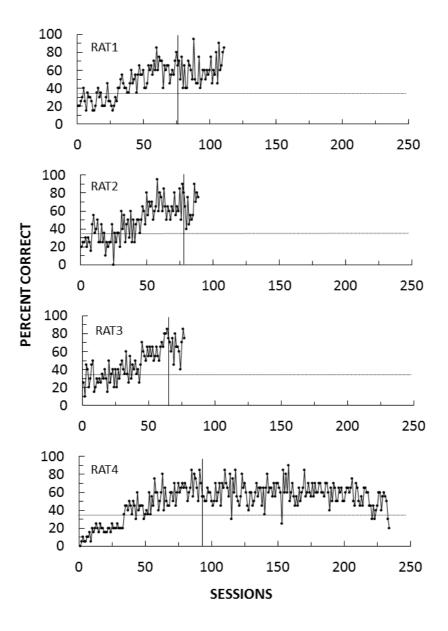
1Makiko Kamijo and Tohru Taniuchi contributed equally to this work.



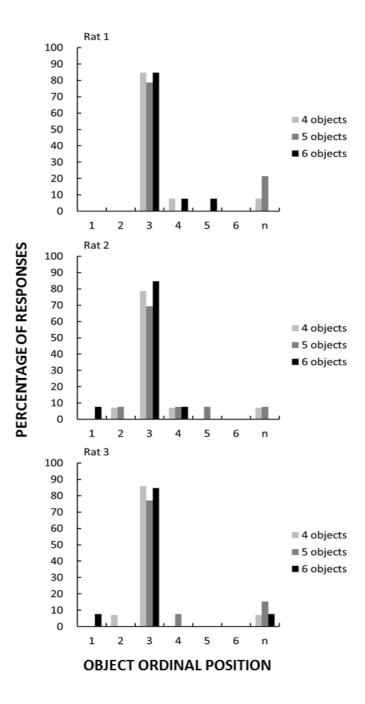
Top shows a schematic representation of the apparatus in the 6-object condition. Correct stimulus was third from left end of object row. Although food rewards were placed in all goal boxes, rats could only access the reward in the correct goal box behind the third object. Bottom is a photograph of the object stimuli used for training.



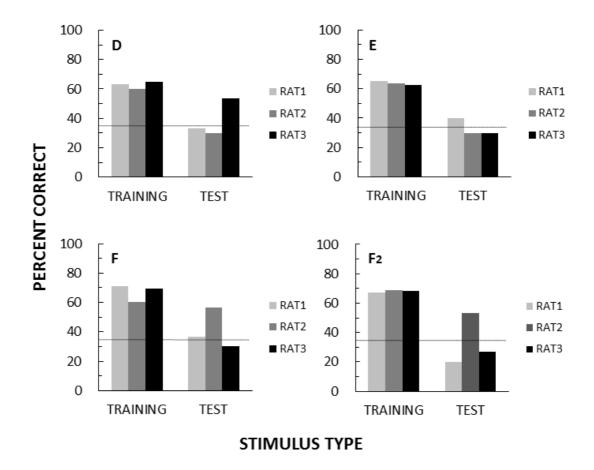
An example of object arrangements in different trials in Training phase 5, in which three kinds of objects, A, B, and C, were used for training. Only one kind of object was used per trial, but the object type was changed every three trials. Total number of objects also varied from 4-6 every three trials.



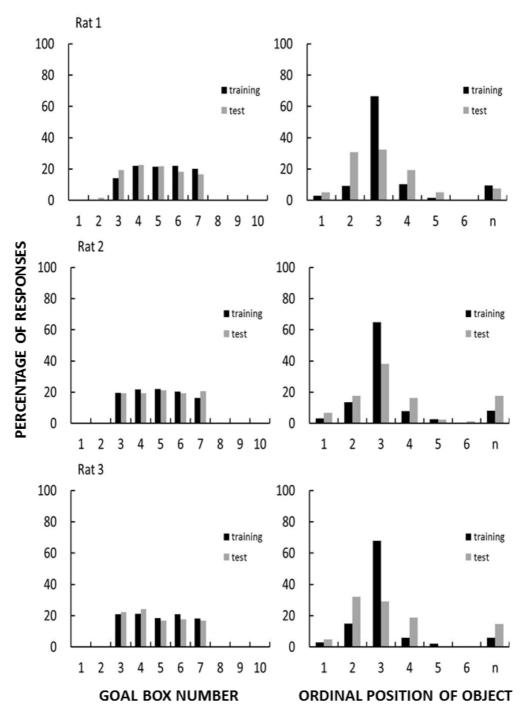
Percentage of correct responses during acquisition training. The solid vertical line represents the beginning of training with three objects. The dotted horizontal line represents the chance level (35.01%).



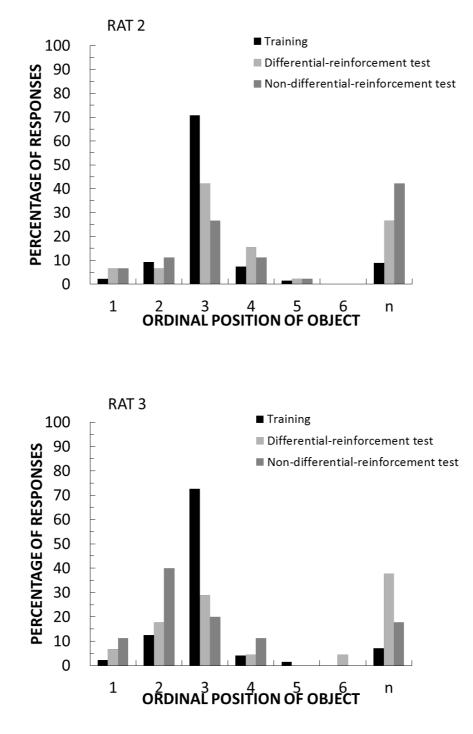
Percentage of responses for each ordinal position of object stimuli averaged over the last two sessions of training phase 5 (objects A, B, and C), training phase 6 (objects A, B, C, and D), and training phase 7 and 8 (objects A, B, C, D, and E). "NO" represents a goal box with no object in front of it.



Percentage of correct responses on each test stimuli in Test phases 1-4. The dotted horizontal line represents the chance level (35.01%). F_2 represents the second probe testing for object F.



Percentage of responses to each goal box (left panel) and to each ordinal position of object stimulus during Test phases 1-4. "NO" represents a goal box with no object in front of it.



Percentage of correct responses for each stimulus type in Test phase 5. The dotted horizontal line represents the chance level (35.01%).