Brief Communication

Same–different categorization in rats

Edward A. Wasserman,¹ Leyre Castro, and John H. Freeman*The University of Iowa, Iowa City, Iowa 52242, USA*

Same–different categorization is a fundamental feat of human cognition. Although birds and nonhuman primates readily learn same–different discriminations and successfully transfer them to novel stimuli, no such demonstration exists for rats. Using a spatial discrimination learning task, we show that rats can both learn to discriminate arrays of visual stimuli containing all same from all different items and transfer this discrimination to arrays composed of novel visual items. These results are consistent with rats' engaging in same–different categorization. As such, they pave the way for investigations into the perceptual, cognitive, and neurobiological substrates of abstract categorization behavior.

[Supplemental material is available for this article.]

Discriminating whether multiple stimuli are the same as or different from one another is a fundamental feat of human cognition, requiring the individual to attend to the relations between the stimuli beyond the particulars of the stimuli (Morgan 1896). Mounting evidence suggests that this cognitive accomplishment is not uniquely human; birds and nonhuman primates readily learn same–different discriminations and successfully transfer them to novel stimuli (e.g., Katz et al. 2007; Wasserman and Young 2010). Rats have not yet been found to learn same–different categorization. Here, we report that rats can learn to discriminate arrays of visual stimuli containing all same from all different items and transfer this discrimination to novel arrays.

The methodological beginning for this work is a study by Wasserman et al. (1978), in which pigeons were trained on an autoshaping procedure that allowed them to associate two successive stimuli with one another. The first stimulus involved either red illumination of the left and right response keys or green illumination of the same two response keys. Following offset of the red lights, a white light appeared on the left key; following offset of the green lights, a white light appeared on the right key (the other half of the pigeons were given the reversed red–right, green–left counterbalancing). Finally, food automatically followed offset of the white light. Pigeons came to peck the left key when the pair of lights was red and to peck the right key when the pair of lights was green (pigeons given the reversed counterbalancing responded in the opposite manner). Clearly, the birds had associated the color of the lights with the location of the upcoming white light.

D Brooks (unpubl. dissertation) further developed this spatial anticipation method by adding a prior step to the stimulus sequence: a central stimulus (Link 1) which preceded the left and right side stimuli (Link 2) and which was the same color as the upcoming pair of stimuli. By deploying touch technology, Brooks measured the spatial location of responses to the central Link 1 stimulus in addition to left–right choices in Link 2. Here, we adapted Brooks' methodology to study rats' same–different discrimination behavior.

Eight rats were given trials containing arrays of 16 identical items (Same trials) or 16 nonidentical items (Different trials). For each Same array, one icon was randomly chosen from a pool of 24 icons and used to create an array of 16 identical items; for each Different array, 16 icons were randomly chosen from the

24-icon pool and used to create an array of 16 nonidentical items (Fig. 1).

As shown in Figure 1, each trial began with a centrally located array (Link 1): either a Same array or a Different array on a random half of the 144 daily training trials. After one response to this array, two arrays were presented on the left and right sides of the screen (Link 2) involving the same inter-stimulus relation as depicted in Link 1. After one response to either of these two arrays, a third stimulus was presented on one side of the screen (Link 3): black concentric rings on a white background. The location of the Link 3 stimulus depended on the type of array presented in Links 1 and 2. For half of the rats, after Same arrays, the Link 3 stimulus appeared on the left, whereas after Different arrays, the Link 3 stimulus appeared on the right. These assignments were reversed for the other half of the rats. After one response to this Link 3 stimulus, food was delivered. Completing the sequence of links always ended with food, regardless of the specific side the rats chose in Link 2. To encourage discrimination learning, a delay was inserted between Link 2 and Link 3 after rats' incorrect choices: responses to the Link 2 array that was in the opposite location from where the Link 3 stimulus was to appear. This delay was initially set at 0 sec and was raised by daily 1-sec steps to a maximum 60 sec. Thus, all trials ended in food reinforcement; correct Link 2 choices resulted in a 0-sec delay, whereas incorrect Link 2 choices resulted in progressively longer delays up to 60 sec (see Supplemental Methods for further details).

Discrimination learning was expected to involve the rats' Link 1 and Link 2 behaviors appropriately anticipating the location of the Link 3 stimulus. Thus, in Link 1, we expected the rats' responses to be located toward the left of the central array when the upcoming Link 3 stimulus would appear on the left and toward the right of the central array when the upcoming Link 3 stimulus would appear on the right. Likewise, in Link 2, we expected the rats to choose the left array when the Link 3 stimulus would appear on the left, and the right array when the Link 3 stimulus would appear on the right.

Training continued for a variable number of sessions for individual rats (from 68 to 91 with a mean of 84.4) before advancement to generalization testing. We used the rats' Link 2 choice accuracy on Same and Different trials as our criterion for advancement. We initially planned on the rats reaching 80% correct in Link 2 on both Same and Different trials before advancement to testing, but only one of the eight rats met that strict criterion. So, after 70 d of training, we adopted four successive criteria: 80% correct on both Same and Different trials (one rat met this

¹Corresponding author.

E-mail ed-wasserman@uiowa.edu.

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Figure 1. The sequence of events in the course of a Same training trial (top) and a Different training trial (bottom). The location of the Link 3 stimulus on Same trials and Different trials was counterbalanced across rats.

criterion); 70% correct, if the 80% criterion was not met after seven more days (three rats met this criterion); 60% correct, if the 70% criterion was not met after seven more days (three rats met this criterion); and, 55% correct, if the 60% criterion was not met after seven more days (one rat met this criterion).

We first analyzed the spatial distributions of rats' responding to the central area in Link 1 during training. We used the x (horizontal)-coordinate of the rats' response locations to the array, which occupied 280 pixels horizontally. For graphing and expository purposes, we aligned the spatial distributions so that the leftmost points (from 0 to 140) corresponded to the upcoming Link 3 location on Same trials and the rightmost points (from 140 to 280) corresponded to the upcoming Link 3 location on Different trials, regardless of the rats' actual stimulus-response assignments (the distributions for half of the rats were thus reversed).

The probability of rats' making a response in 28-pixel bins across the central array over the last 10 training sessions is shown in Figure 2 (top left). The rats were much more likely to respond to the Same (left) side of the central array when the Link 3 stimulus would next be located on the left; conversely, the rats were much more likely to respond to the Different (right) side of the central array when the Link 3 stimulus would next be located on the right (see each rat's response frequencies in Supplemental Table S1).

A 2 (trial type: Same vs. Different) $\times$ 10 (bin) $\times$ 10 (session) repeated-measures analysis of variance (ANOVA) on Link 1 response probabilities over the last 10 training days yielded a significant Trial Type $\times$ Bin interaction, $F_{(9,63)} = 235.56$, $P < 0.001$, confirming rats' appropriate anticipatory behavior when the Same or the Different array was presented on the center screen. There was no significant effect of session, nor did session interact with any other factor (all $F_s < 1$). Thus, there were no systematic changes in Link 1 behavior across the last 10 training sessions (see Supplemental Table S2).

Next, we examined the percentage of correct choices in Link 2. Over the final 10 d of training, mean Link 2 accuracy was 72%

(Fig. 2, top right). Mean accuracy was 65% on Same trials and 78% on Different trials, both reliably higher than chance (50%; one-sample $t_{(7)} = 11.9$, $P < 0.001$, and $t_{(7)} = 31.3$, $P < 0.001$, for Same and Different trials, respectively). A 2 (trial type: Same vs. Different) $\times$ 10 (session) repeated-measures ANOVA yielded a marginally significant disparity between Same and Different trials, $F_{(1,7)} = 5.4$, $P < 0.06$. There was no significant effect of session nor did session interact with trial type (all $F_s < 1$). Thus, there were no systematic changes in accuracy across the last 10 training sessions (see Supplemental Table S3).

Despite the difficulty of the task, rats did exhibit reliable same-different discrimination in both Link 1 and Link 2. Link 1 responses were appropriately directed to the left or right regions of the central array depending on whether Same or Different arrays were presented; as well, Link 2 responses were appropriately directed to the left or right arrays depending on whether Same or Different arrays were presented. The rats did respond more accurately on Different trials than on Same trials in Link 2. This Different advantage has been reported for pigeons given other training techniques (Wasserman et al. 2002; Blaisdell and Cook 2005; Castro et al. 2010). Possible reasons for this Different advantage are discussed by Castro et al. (2010).

Beyond acquisition, we also expected the rats to reliably transfer Link 1 and Link 2 discrimination behavior to Same and Different arrays created from novel items, thereby documenting the generality of the rats' categorization (Delius 1994).

Generalization testing comprised 20 sessions of 176 trials: 144 trials in each session were baseline training trials, and 32 were novel testing trials. Testing trials contained arrays with novel items selected from a new 16-icon pool. Beyond the novelty of the icons, testing trials were unlike training trials in two more respects. First, the Link 3 stimulus was always located on the same side of the screen that the rat had chosen in Link 2. Second, there were no delays to Link 3 presentations following Link 2 responses. These two changes ensured that we were not teaching the rats to make the "correct" responses to the testing stimuli. In order to analyze Link 2 testing choices, we deemed to be correct the left or right choice that was correct on Same and Different training trials.

In generalization testing, both Link 1 response probability scores (Fig. 2, bottom left; see each rat's responses in Supplemental Tables S4, S5) and Link 2 choice accuracy scores (Fig. 2, bottom right) disclosed virtually identical responding to the training and testing stimuli.

A 2 (stimuli: training vs. testing) $\times$ 2 (trial type: Same vs. Different) $\times$ 10 (bins) $\times$ 5 (blocks of four daily sessions) ANOVA on the rats' Link 1 response probabilities yielded a significant Trial Type $\times$ Bin interaction, $F_{(9,63)} = 358.22$, $P < 0.001$, confirming rats' appropriate anticipatory behavior in Link 1. There was also a Trial Type $\times$ Bin $\times$ 4-Session Block interaction, $F_{(36,252)} = 1.96$, $P < 0.01$, a Trial Type $\times$ Bin $\times$ Stimuli interaction, $F_{(9,63)} = 2.75$, $P < 0.01$, and a Trial Type $\times$ Bin $\times$ Stimuli $\times$ 4-Session Block interaction, $F_{(36,252)} = 1.55$, $P = 0.02$. Rats' appropriate anticipatory behavior was evident to both training and testing stimuli in all five testing blocks (see Supplemental Table S6), with nonsystematic fluctuations contributing to these statistical interactions.

Link 2 choice accuracy on Same trials to the training stimuli and the testing stimuli averaged 67% and 66%, respectively, over the 20 d of generalization testing; both scores were reliably higher than chance (50%; one-sample $t_{(7)} = 19.6$, $P < 0.001$ and $t_{(7)} = 15.6$, $P < 0.001$, respectively, for training and testing stimuli). As well, Link 2 choice accuracy on Different trials to the training and testing stimuli averaged 77% and 80%, respectively; both scores were reliably higher than chance (50%; one-sample $t_{(7)} = 32.9$, $P < 0.001$, and $t_{(7)} = 27.4$, $P < 0.001$, respectively, for training and testing stimuli).

Figure 2. (Top left) Response probability across the entire width of the Link 1 stimulus during the last 10 training days. The x-axis represents the 280-pixel width of the Link 1 stimulus divided into 10 28-pixel bins. The spatial distributions were aligned so that the location of upcoming Link 3 was always left for Same trials and right for Different trials, regardless of the rats' actual stimulus-response assignments (see text and Supplemental Methods for details). (Top right) Mean percentage of correct choices in Link 2 for Same and Different trials during the last 10 training days. (Bottom left) Response probability across the entire width of the Link 1 stimulus during the 20 d of generalization testing. (Bottom right) Mean percentage of correct choices in Link 2 for Same and Different trials during the 20 d of generalization testing.

A 2 (stimuli: training vs. testing) $\times$ 2 (trial type: Same vs. Different) $\times$ 5 (blocks of four daily sessions) ANOVA of Link 2 choice accuracy scores disclosed that Different trials (78%) supported more accurate responding than Same trials (67%), $F_{(1,7)} = 147.05$, $P < 0.001$; as well, there was a significant Stimuli $\times$ Trial Type interaction, $F_{(1,7)} = 4.18$, $P = 0.04$, suggesting that the disparity between Same and Different trials was greater on testing trials (66% vs. 80%) than on training trials (67% vs. 77%). This pattern was present the very first testing day (accuracy on testing trials was 57% and 83% for Same and Different trials, respectively; accuracy on training trials was 61% and 79% for Same and Different trials, respectively). There was also a significant Trial Type $\times$ 4-Session Block interaction, due to accuracy for Same trials increasing toward the end of testing (see Supplemental Table S7); indeed, the disparity in accuracy between Same and Different trials was not significant in the last testing block ($F < 1$). Nevertheless, there was no overall reliable disparity in Link 2 choice accuracy between training (72%) and testing trials (73%), $F_{(1,7)} = 1.59$, $P > 0.1$.

Clearly, changing items within the Same and Different arrays had no detrimental effect on rats' Link 1 and Link 2 discriminative responding; rats' performance was equally robust on novel testing trials and familiar training trials. Thus, in addition to learning to

respond discriminatively to arrays of same and different visual items, the rats reliably transferred these learned responses to arrays created from novel visual items. Such robust acquisition and generalization is consistent with the rats' successful same-different categorization, much like the pigeons, baboons, and humans given similar experimental tasks (Wasserman and Young 2010); however, more research will be needed to determine whether all of these species perform various categorization tasks in the same way.

Our findings pave the way for follow-up studies aimed at elucidating the perceptual, cognitive, and neurobiological substrates of abstract categorization behavior, especially the neural structures and systems that mediate same-different categorization behavior. Previous studies with monkeys have found that neurons in the prefrontal (PFC) and premotor (PMC) cortex selectively code for Same and Different rules, in which a response was associated with matching trials or nonmatching trials, depending on the initial sample cue (Wallis and Miller 2003; Muhammad et al. 2006). A subset of PFC and PMC neurons showed differential activity for Same and Different trials but not for particular cues or for matching vs. non-matching trials. The sample stimuli were single complex objects rather than arrays, so the monkeys were not required to assess the relations among multiple objects within a display. Our procedure required rats to assess the relations among multiple items within a display to determine sameness or differentness. Encoding the relations among multiple stimuli and forming relational representations

have been hypothesized to be functions of the hippocampus (Ryan et al. 2000; Eichenbaum and Cohen 2001). The ability to assess the relations among multiple items may thus be particularly dependent on hippocampal processing.

We plan to conduct neurobiological studies to examine the respective roles of the rodent prefrontal cortex and hippocampus in same-different categorization behavior.

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References

- Blaisdell AP, Cook RG. 2005. Two-item same-different concept learning by pigeons. *Learn Behav* **33**: 67–77.
- Castro L, Kennedy PL, Wasserman EA. 2010. Conditional same-different discrimination by pigeons: Acquisition and generalization to novel and few-item displays. *J Exp Psychol Anim B* **36**: 23–38.
- Delius JD. 1994. Comparative cognition of identity. In *International perspectives on psychological science* (ed. P Bertelson et al.), pp. 25–40. Erlbaum, Hillsdale, NJ.

- Eichenbaum H, Cohen NJ. 2001. *From conditioning to conscious recollection: Memory systems of the brain*. Oxford University Press, London, UK.
- Katz JS, Wright AA, Bodily KD. 2007. Issues in the comparative cognition of abstract-concept learning. *Compar Cogn Behav Rev* **2**: 79–92.
- Morgan CL. 1896. *An introduction to comparative psychology*. Walter Scott Ltd, London, UK.
- Muhammad R, Wallis JD, Miller EK. 2006. A comparison of abstract rules in the prefrontal cortex, premotor cortex, inferior temporal cortex, and striatum. *J Cognitive Neurosci* **18**: 974–989.
- Ryan JD, Althoff RR, Cohen NJ. 2000. Amnesia is a deficit in relational memory. *Psychol Sci* **11**: 454–461.
- Wallis JD, Miller EK. 2003. From rule to response: Neuronal processes in the premotor and prefrontal cortex. *J Neurophysiol* **90**: 1790–1806.
- Wasserman EA, Young ME. 2010. Same-different discrimination: The keel and backbone of thought and reasoning. *J Exp Psychol Anim B* **36**: 3–22.
- Wasserman EA, Carr DL, Deich JD. 1978. Association of conditioned stimuli during serial conditioning by pigeons. *Anim Learn Behav* **6**: 52–56.
- Wasserman EA, Frank AJ, Young ME. 2002. Stimulus control by same versus different relations among multiple visual stimuli. *J Exp Psychol Anim B* **28**: 347–357.

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