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Visual Memory of Shapes in Quail Chicks: Discrimination among 2-Dimensional Objects

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ABSTRACT—Newly hatched chicks spontaneously peck at conspicuous objects, and soon learn to discriminate between edible food particles and inedible objects. To examine whether this discrimination is based on a chick's ability to memorize objects by shape cues, we analyzed the pecking behavior. One- to 3-day old quail chicks (*Coturnix japonica*) were presented with dry objects of different shapes (*ball, disk, triangle* and *T-shape*) of similar size (4 mm) and color (green). Habituation occurred after repeated presentation of any one of these objects (duration: 30 sec; interval: 4 min). When chicks showed significantly more pecks at a novel object (dishabituation), we assumed that chicks had memorized the habituated shapes and distinguished the novel object. Chicks did not show dishabituation between a *ball* and a *disk*. On the other hand, chicks discriminated a *triangle* or *T-shape* from the memorized image of *disk*, but did not memorize either *triangle* or *T-shape* by its shape. Similarly, chicks did not memorize the size of disks as a reference for subsequent pecking behavior. Chicks proved to have a limited ability to memorize shape and size cues for selective pecking behavior, in strong contrast to their accurate memorization of colors.

Keywords: bird, memory, imprinting, passive avoidance, reinforcement learning

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INTRODUCTION

Newly hatched chicks of precocial birds have an innate tendency to peck at small conspicuous objects such as bright and colorful beads. When the object is edible or positively rewarding, the chick will subsequently show enhanced pecking that is often followed by biting and swallowing. When the object is not edible or not rewarding at all, the chick will become habituated to the object and gradually cease pecking. When the object tastes bitter, on the other hand, the chick will learn to avoid similar objects after a single experience of the bad-tasting bead. A variety of learning tasks have been developed based on these tendencies of chicks, i.e., one-trial passive avoidance task (Cherkin 1969, Rose SPR 1991), visual habituation (Aoki *et al*. 2000, Sakai *et al*. 2000), and rewarded GO / NO-GO task (Yanagihara *et al*. 2001).

Color is thought to be one of the dominant cues for the visual categorization of objects. Osorio and his colleagues (1999) have shown that chicks form accurate memories of color. In the passive avoidance learning paradigm, chicks precisely memorized the coloration of a bitter tasting bead even without any differential pre-training experiences (Aoki *et al*. 2000). Similar precision in the memorization of color has been documented with a water-reinforced GO pecking task, where chicks learned the cues of appetizing objects (Izawa *et al*. 2001).

Other non-color cues have also been suggested in chicks. A localized brain lesion is reported to cause color blindness, while preserving chicks' ability to avoid the bitter-tasting bead (Patterson and Rose 1992). The ablated region (intermediate medial hyperstriatum ventrale, or IMHV) in the dorsal telencephalon is thought to be critical in both passive avoidance task (Rose SPR 1991) and imprinting (Horn 1985, Horn 1998)). Patterson and Rose (1992) have argued that the IMHV is responsible specifically for the visual association of colors and selective avoidance, whereas the ablated chicks could avoid the bitter-tasting object on the basis of non-color cues such as shape or size. However, so far there has been no evidence directly supporting that chicks memorize shape cues for the visual categorization of objects. The purpose of the present study was to determine whether and how shapes could serve as significant visual cues for chicks to recognize objects. The results of this study support our preliminary data (Sakai *et al*. 2000), which suggested that chicks are innately predisposed to memorize a limited class of shapes such as balls or disks, and discriminate other shapes in reference to these memorized circular images.

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MATERIALS AND METHODS

Subjects and housing conditions

Newly hatched quail chicks (*Coturmix japonica*) were used. Fertilized eggs were obtained from a commercial supplier, and incubated in a dark incubator kept at 37.7°C. Chicks were left in the incubator for about 24 hr after hatching. Afterwards, healthy chicks were labeled with leg rings and randomly paired in small transparent plastic cages (13×9 cm^2, 9 cm high). The cages were kept in a breeder that was illuminated by dim white light (35–80 lux, 12 L: 12 D), highly moisturized and kept at a controlled temperature between 31–35°C. The chicks were given a petri dish containing a water-soaked sponge and allowed to drink freely. From 48 hr post-hatch, chicks were also given quail mash food. In this study, experiments were performed at 24–72 hr post-hacth.

Visual objects for discrimination

Six objects were used, i.e., 4 of different shapes and 2 of different sizes (Fig.1 C). Objects were presented in the orientation shown in Fig. 1C, and not rotated along the bar axis. The surface of objects was painted green (type X-28 acrylic paint; Tamiya Co., Japan), and the objects were glued to transparent plastic rods (1.5 mm in diameter). The ball was a 3-dimensional plastic sphere. A *disk, triangle* and *T-shape* were made of 1.0 mm thick acrylic film. When compared among these shapes, number of pecks did not significantly differ upon the first encounter (1st trials in Fig. 3A, B, and C), thus suggesting that chicks did not have an innate biased to peck at specific shapes. Disks of two other sizes, one smaller (× *1/2 disk*, or 2.8 mm in diameter) and another larger (× *2 disk*, or 5.6 mm in diameter) were also made of the same film. One of these objects was presented through a hole on the wall. The orientation of the objects was fixed as illustrated in Fig. 1C, and not rotated along the rod axis. Note that the *ball* gives an invariant disk image on the retina irrespective of the chick's viewpoint, while the image of the other objects would vary depending on the angle from which they are viewed. The surface area of these objects was as follows; *disk*: 12.6 mm^2, *triangle*: 6.9 mm^2, *T-shape*: 7.0 mm^2, × *1/2 disk*: 6.2 mm^2, and × *2 disk*: 24.6 mm^2, respectively.

General procedure of tests

The subject chick was always paired with another "soother" chick to suppress distress calling by the subject (Hayashi *et al.* 2001). During experiments, the pair was placed in a chamber (15×11 cm^2, 18.5 cm high) within a cabinet (Fig.1A). One sidewall of the cabinet was equipped with a transparent acrylic window. The inside of the cabinet was illuminated (ca. 1,080 lux) and thermo-controlled at 30±1°C (Fig. 1C). Before each session, the chicks were given 3 min in the box for acclimation. The subject was then separated from the "soother" chick by a transparent acrylic plate, and left for another 3 min. The plate was used in order to suppress social facilitation of pecking by the "soother". The subject chick was thereafter examined for its preference in pecking behavior. Test objects were presented at 4 min intervals. The object was left for up to 4 min until the chick turned to or approached the object; if a chick failed to turn to / approach the object, it was excluded. The number of pecks was counted during a 30 sec period starting from the first turn or approach. Objects were manipulated by an experimenter, and presented by hand through a small hole (7.0 mm in diameter) in the wall of the chamber located 3.0 cm above the floor. Dry objects were used in all experiments. The objects were presented one at a time. The object was in a fixed orientation, and not rotated along the rod axis. The behavior of the chicks was observed through a Plexiglas one-way window of the chamber. Mann-Whitney's U-test was used to compare two independent sets of data.

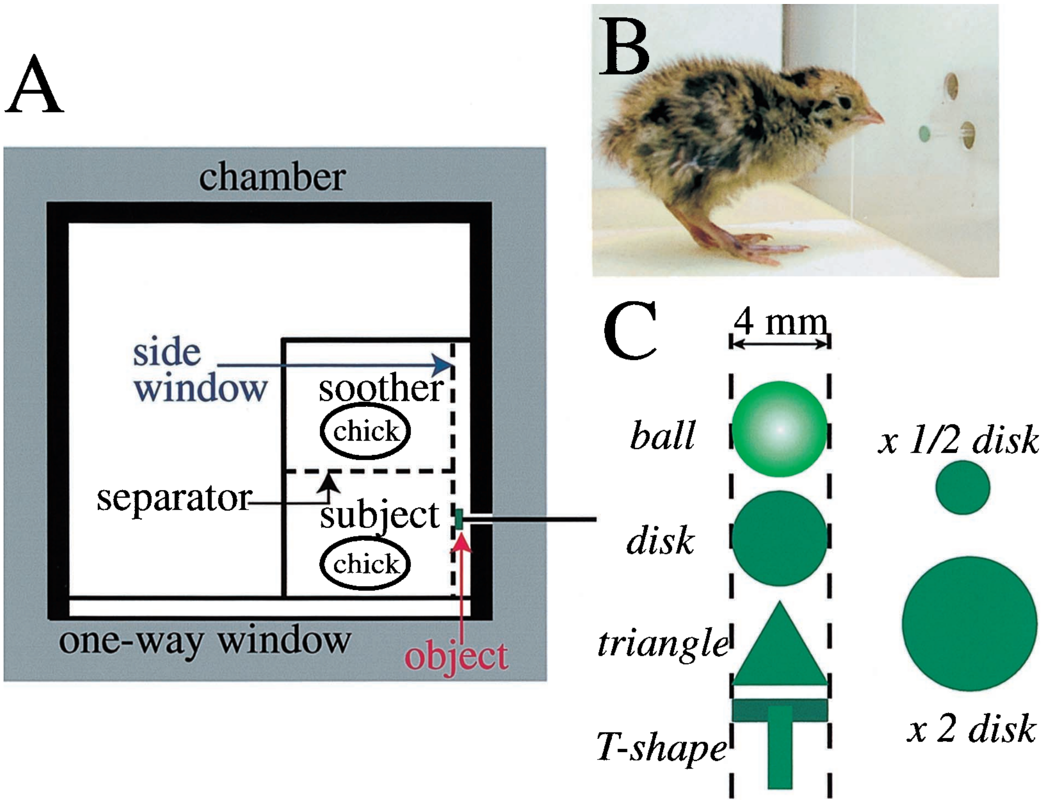

Fig. 1. A: Schematic illustration of the experimental setup. B: Photograph of a subject quail chick. C: Frontal views of objects used in this study.

Sign test was used to compare the actual number of pecks (the 4th [Fig. 2, 3, 5], or the 7th [Fig. 4]) with expected number (average of the 3rd and the 5th [Fig. 2, 3, 5], or average of the 6th and the 8th [Fig. 4]). The level of significance was set at 0.05 or below.

RESULTS

Chicks did not visually discriminate between ball and disk

What mental images could chicks make upon pecking at a 3-dimensional *ball*? On the retina, the *ball* may give a disk-shaped image similar to the 2-dimensional *disk*. Otherwise, chicks may discriminate between these two objects based on differences such as surface shading and texture. Another possibility is that chicks rely on the tactile sensation caused by direct touch of the beak, and distinguish objects of different physical properties. The following three experimental groups were designed in order to differentiate among these possibilities.

Chick pairs were randomly assigned into 3 groups, and subject chicks were given 5 successive presentations. The same object was presented in the 1st to the 5th trials, except for the 4th trial when a different object was presented. These 3 groups are as follows:

Group 1: *ball* - *ball* - *ball* - *disk* - *ball* (without the side window)

Group 2: *ball* - *ball* - *ball* - *disk* - *ball* (with the side window)

Group 3: *disk* - *disk* - *disk* - *ball* - *disk* (with the side window)

Without the side window, chicks pecked directly at the object, and often bit and pulled it in attempts to eat it. With the side window, on the other hand, chicks did not peck at the objects directly. Instead, they pecked at the point on the acrylic plate where the test object was presented. No specific tactile cues could be obtained (see Fig. 1B). In addition, the side window prevented chicks from approaching and watching objects from the side, so that the retinal image of the object remained relatively unvaried.

Without the side window (group 1), chicks habituated to a *ball* from the 1st to the 3rd trials, and showed a dishabituated pecking at a *disk* (the 4th trial in Fig. 2A), suggesting that chicks discriminate between *ball* and *disc*. With the side window, therefore without any specific tactile cues available, chicks showed a similarly habituated pecking from the 1st to the 3rd trials. However, they did not show a dishabituation to the novel object presented in the 4th trial (group 2 and 3) (Fig. 2B). A comparison of groups 2 and 3 revealed no significant difference in the number of pecks between corresponding trials (1st, 2nd, 3rd, and 5th), suggesting no biased preference between the *ball* and *disk*. Chicks might have ignored 3-dimensional visual cues such as binocular disparity and surface texture of the objects. The 2-dimensional visual cue of the outline may thus be critical for visual discrimination of objects. In the next experiment, we examined whether chicks could discriminate between a circular object (*disk*) and other objects with distinct outlines (*triangle* and *T-shape*).

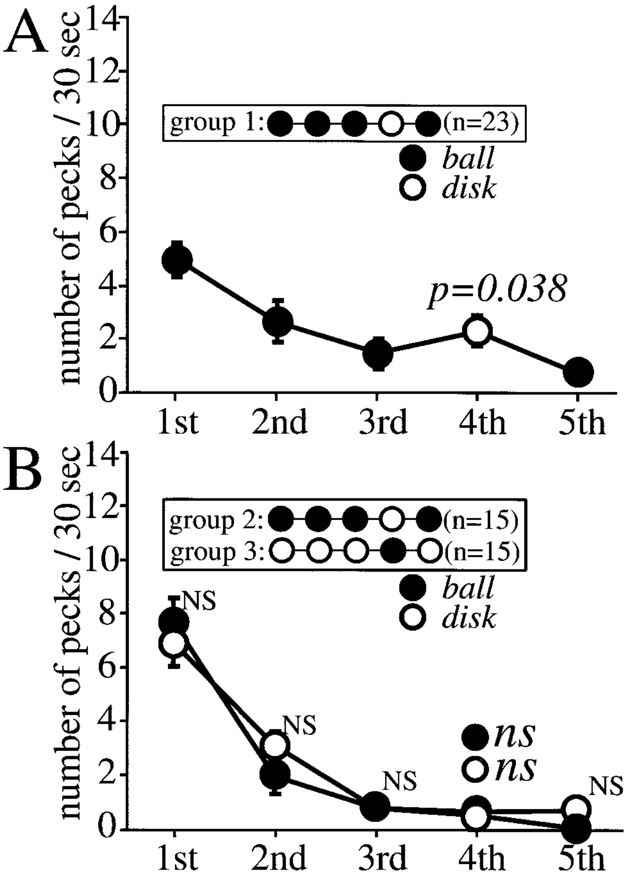

Fig. 2. With visual cues alone, chicks did not discriminate between the 3-dimensional *ball* and the 2-dimensional *disk* of the same size. The number of pecks (ordinate; mean±S.E.M.) during 30 sec was plotted against the trial number (abscissa). The presentation procedure is illustrated in the inset diagrams. Filled circles denote *ball*, and open circles *disk*. The *n* indicates the number of chick pairs used in each group. A: Without the transparent side window, chicks showed significant dishabituation to the *disk* after being habituated to the *ball*. The number of pecks in the 4th trial was significantly greater than the expected value (average of the 3rd and the 5th) with p=0.038. B: With the side window, however, chicks did not show dishabituated pecking in either group. No significant difference was found compared with the expected value (i.e., average of the 3rd and the 5th trials) (*ns*); Signed rank test (p>0.05). No significant difference was found between group 2 and 3 (*NS*); Mann-Whitney's U-test (p>0.05). Results of similar statistical tests are also shown in the following figures.

Chicks discriminated triangle and T-shape from a memorized image of disk

In the next step, we examined the discrimination between *disk* and *T-shape* (Fig. 3A) in the following 2 groups:

Group 4: *disk* - *disk* - *disk* - *T* - *disk*

Group 5: *T* - *T* - *T* - *disk* - *T*

In this and the following experiments, the subject chick was separated from the object by a side window. After being

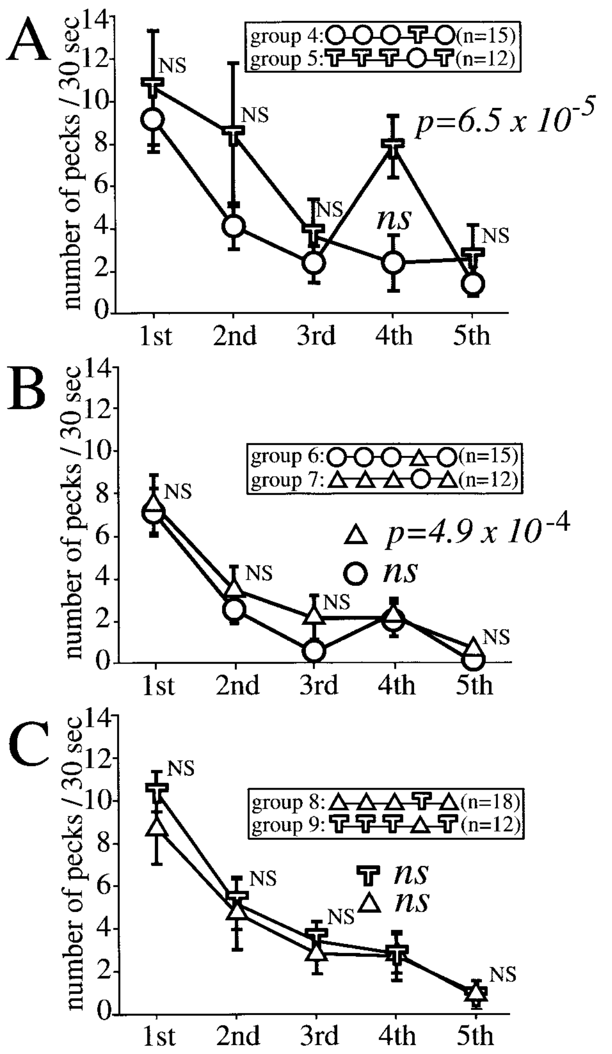

Fig. 3. Chicks discriminated the *triangle* and *T-shape* from the *disk*. A: After being habituated to the *disk*, chicks showed significant dishabituation to the *T-shape* (group 4). In group 5, where an opposite combination was examined, chicks failed to show dishabituation to the *disk* after the *T-shape* (*ns*). Between the two groups, the number of pecks did not differ between corresponding trials (*NS*; 1st, 2nd, 3rd, and 5th). B: Similar results were obtained between the *disk* and *triangle*, although the degree of dishabituation was weaker for the *triangle* than the *T-shape* shown in A. C: Chicks did not show dishabituation between the *triangle* and *T-shape* (*ns*).

habituated to a *disk*, chicks showed a clear dishabituation to a *T-shape* (group 4). However, in group 5 where an opposite combination was examined, chicks did not show dishabituation to a *disk*. No biased preferences were found between the two objects in the 1st to the 3rd, and the 5th trials. The apparent asymmetry between these objects suggests that chicks can memorize the *disk* shape and distinguish the *T-shape*, whereas they can not memorize the T-shape object by its shape.

Similar results were obtained between *disk* and *triangle* (Fig. 3B) in 2 other experimental groups:

Group 6: *disk - disk - disk - triangle - disk*

Group 7: *triangle - triangle - triangle - disk - triangle*

A comparison between groups 4 and 6 showed that the degree of dishabituation in the 4th trial was much weaker for the *triangle* (group 6) than the *T-shape* (group 4) (Mann-Whitney's U-test: $p < 0.01$). The *T-shape* might be perceived as more distinctively different than the *triangle* from the *disk*.

We compared the triangle and *T-shape* (Fig. 3C) directly in the following 2 groups:

Group 8: *triangle - triangle - triangle - T - triangle*

Group 9: *T - T - T - triangle - T*

Dishabituation did not occur in either group. Under these conditions, chicks did not discriminate between these two objects, both of which were composed of straight lines and edges. How do chicks distinguish between the *triangle* and the *T-shape*, if chicks were tested after they had successfully discriminated these objects from the *disk*? Once they have learned to discriminate objects by shape, chicks might pay specific attention to the shape cue. In the next experiment, we thus examined the effects of preceding experiences.

Chicks discriminated T-shape from triangle, once they were habituated to disk

Chicks were initially habituated to a *disk* in 3 successive trials, and then tested according to the same procedures as in groups 8 and 9. Eight successive presentations were thus given. These groups are as follows:

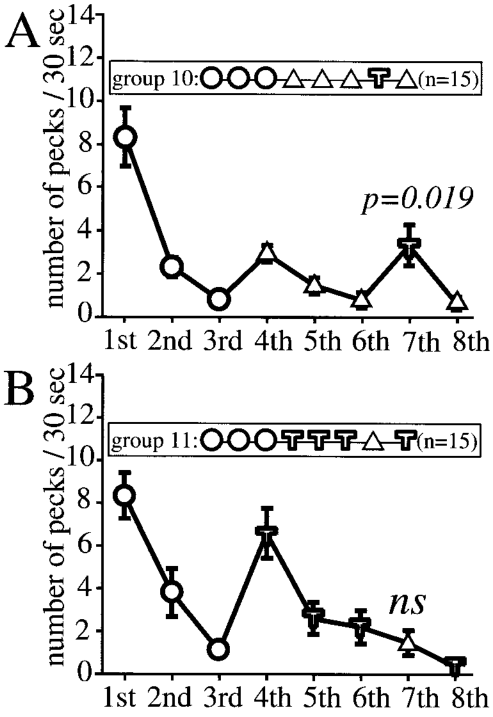

Fig. 4. Effects of prior experiences of the *disk* on the discrimination between the *triangle* and *T-shape*. A: After habituation to the *triangle* in the 4th to the 6th trials, chicks showed a significant dishabituation to the *T-shape* in the 7th trial (p=0.019). B: After habituation to the *T-shape*, chicks failed to show a dishabituation to the *triangle* in the 7th trial (*ns*).

Group 10: *disk - disk - disk - triangle - triangle - triangle - T - triangle*

Group 11: *disk - disk - disk - T - T - T - triangle - T*

In group 10 (Fig. 4A), chicks clearly showed dishabituation to the *T-shape* in the 7th trial, in contrast to group 8 (Fig.3C). In group 11 (Fig. 4B), on the other hand, chicks did not show significant dishabituation to the *triangle* after the *T-shape*, similar to the findings with group 9. In the 4th trial, i.e., after 3 successive trials using the *disk*, chicks showed significantly more pecks at the *triangle* (group 10) than the *T-shape* (group11) (p = 0.012; Mann-Whitney's U-test).

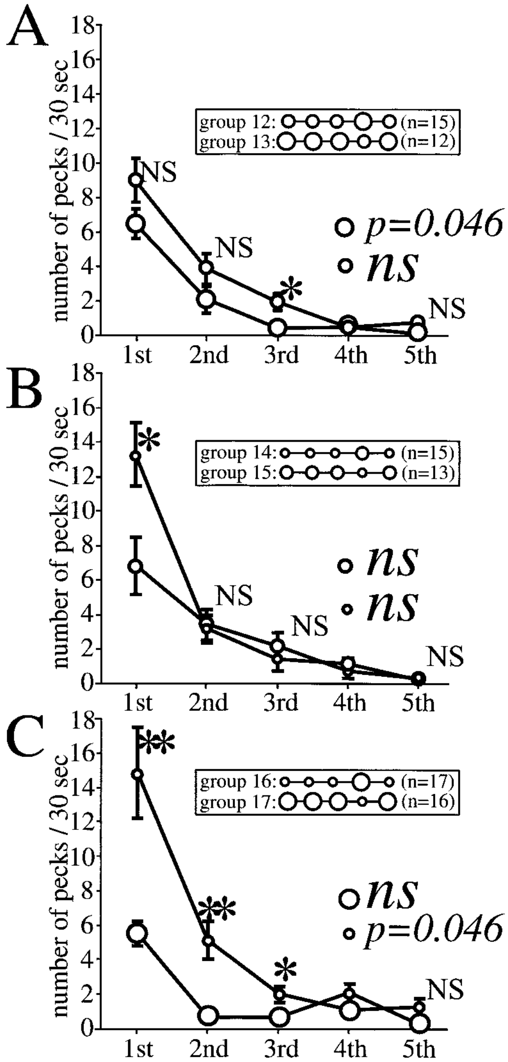

Fig. 5. Chicks discriminated among disks of different sizes, though loosely. A: Dishabituation did not occur between the *disk* and × *2 disk*. B: There was also no dishabituation between the × and × *1/2 disk*. C: After being habituated to the × *2 disk*, chicks showed dishabituated pecking at the × *1/2 disk*. However, chicks did not show dishabituation to the × *2 disk* after the × *1/2 disk*. Note that smaller objects tended to induce more pecks in the first trial. Comparisons between groups by Mann-Whitney's U-test; *: $p < 0.05$, **: $p < 0.01$, *NS*: $p \geq 0.05$.

Chicks discriminated objects of different sizes, though they did not memorize the size as a significant cue

Both the *triangle* and *T-shape* had a smaller surface area than the *disk*, and chicks might have discriminated between these objects not by their shapes, but by the different sizes. As the final step in this series of experiments, we examined whether a size cue could be critical. To avoid complications, disks of 3 different sizes were used in the following 6 experimental groups:

Group 12: *disk - disk - disk -* × *2 disk - disk*

Group 13: × *2 disk -* × *2 disk -* × *2 disk - disk -* × *2 disk*

Group 14: × *1/2 disk -* × *1/2 disk -* × *1/2 disk - disk-* × *1/2 disk*

Group 15: *disk - disk - disk -* × *1/2 disk - disk*

Group 16: × *1/2 disk -* × *1/2 disk -* × *1/2 disk -* × *2 disk-* × *1/2 disk*

Group 17: × *2 disk -* × *2 disk -* × *2 disk - 1/2 disk -* × *2 disk*

In groups using the *disk* and × *2 disk* (groups 12 and 13), dishabituation did not occur irrespective of the order of presentations (Fig. 5A). In the 4th trial of group 12 (× *2 disk*), chicks pecked significantly less than the average of the 3rd and the 5th trials (p=0.046). This is probably due to the significantly different habituation to these objects: the asterisk in the 3rd trial between groups 12 and 13 (Fig. 5A). Similar results were obtained between the × *1/2 disk* and disk (groups 14 and 15), as shown in Fig. 5B. In the 1st trial, chicks pecked significantly more at the × *1/2 disk* than at the *disk*, indicating a clear preference for smaller objects. Between the × *1/2 disk* and × *2 disk* (groups 16 and 17), similarly, a clear dishabituation did not occur (Fig. 5C). After repeatly pecking at the × *1/2 disk*, chicks did not show dishabituation at the × *2 disk*; however, dishabituation occurred with the × *1/2 disk* after the × *2 disk*. In the 1st to the 3rd trial, the group 17 chicks pecked more than the group 16 chicks, again indicating chicks' preference for smaller objects.

DISCUSSION

The results obtained in this study are summarized in Fig. 6. Using visual cues alone, chicks did not discriminate between the *ball* (3-D object) and *disk* (2-D object), both of which should give rise to a similar circular image on the retina (Fig. 2). Chicks could distinguish the other shapes (*triangle* and *T-shape*) from the memorized image of the *disk* (Fig. 3A, B). Most probably, chicks were concerned with whether the outline of the object was composed of curves (*disk*) or lines and edges (*triangle* and *T-shape*). When the *triangle* and *T-shape* were compared directly, however, chicks did not show dishabituated pecking (Fig. 3C). On the other hand, when tested after being habituated to the *disk*, chicks showed dishabituated pecking at the *T-shape* after the *triangle* (Fig. 4A), but not with the reverse order (Fig. 4B). Finally, chicks distinctively pecked at disks of different sizes (Fig. 5). All the results obtained in the present study

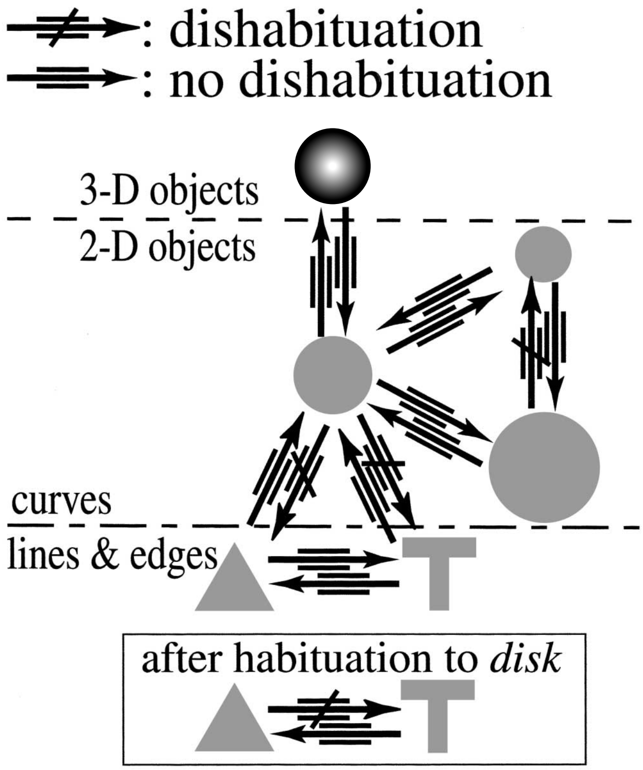

Fig. 6. Summary of the present experiments, schematically illustrating the visual categorization of shapes in quail chicks. Note the asymmetric generalization between the categories “curves” and “lines & edges.” See text for further explanations.

basically support the findings of the previous report (Sakai *et al*. 2000), indicating that chicks’ ability to recognize shapes is limited in comparison with their ability to recognize colors (Aoki *et al*. 2000). When discriminating colors, the degree of habituation accurately reflected the physical differences among colors. Furthermore, chicks discriminated green from yellow, and similarly yellow from green; color discrimination proved symmetric. As far as the pecking behavior is concerned, the color cues are much more critical than the shape cues.

Did chicks directly discriminate between the triangle and *T-shape*?

As shown in Fig. 4A, the preceding experiences of pecking at the disk affected the following discrimination between the *T-shape* and *triangle*. Without such a preceding experience, chicks did not discriminate between these shapes (Fig. 3C). The discrepancy between these two experiments (groups 8 and 10) may be interpreted in either one of the following ways. (1) After chicks learned to discriminate the *triangle* from the memorized image of the *disk*, they became more attentive to the difference in shape cues, and thus could directly discriminate the *T-shape* from the memorized image of the *triangle*. Alternatively, (2) chicks could memorize only the shape of the *disk*, and compared the two shapes (*triangle* and *T-shape*) in reference to the memorized image of the *disk*. To the chicks’ eyes, the *T-shape* might have seemed more distinctive from *disk* than the *triangle* did. Actually, the stronger dishabituation appeared with the *T-shape* than the *triangle* (4th trials in group 4 vs. group 6; 4th trials in group 11 vs. group 10), thus favoring the latter interpretation. On the other hand, we do not have any lines of evidence, independently supporting chick’s ability to memorize the *triangle* shape.

Studies of filial imprinting report that chicks can memorize and discriminate between different shapes such as a box and cylinder (Horn 1985). It has also been shown that domestic chicks can be imprinted with a cardboard triangle (Regolin and Vallortigara 1995). Definitely, chicks have the capacity to memorize complex shapes, and results obtained using the imprinting paradigms are contradictory to the present results. The difference in object sizes might explain the discrepancy; the objects used in imprinting (5–20 cm) are much bigger than the objects used in this study (2–6 mm), so that chicks could memorize the shape much more easily. Alternatively, a difference in the behavioral paradigm might explain the discrepancy; chicks might form a detailed shape image of the imprinting object, but simply do not care about the shape of objects they peck at. Otherwise, the difference in the training time might explain the different results. In imprinting, chicks are usually exposed to objects for a period of 1 to 2 hr. On the other hand, chicks might stare at objects they peck at for only a few seconds to a minute.

Did chicks directly discriminate between different sizes of disk?

As shown in group 17 (Fig. 5C), chicks showed a dishabituated pecking at the × *2 disk* after being habituated to the × *1/2 disk*; the size ratio was 1:4. It is also evident that chicks have an innate preference for smaller objects over larger ones (Fig. 5B, 5C). These results can be interpreted in the following two ways. (1) Chicks memorized the size of object they pecked, and discriminated among objects of different size based on the memorized size. Or, (2) Chicks behaved differently when presented with different size objects, simply because they preferred smaller objects. If the former is correct, we could expect a dishabituated pecking also at a × *2 disk* after being habituated to a × *1/2 disk*. However, this was not the case, and chicks failed to show a dishabituation (group 16). Although chicks may have an internal (mental) scale for measuring the size of objects, chicks do not memorize the size they measured.

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