SHORT COMMUNICATION

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Visual discrimination of normal and drug induced behavior in quails (*Coturnix coturnix japonica*)

Abstract The ability to discriminate the physical states of others could be an adaptive behavior, especially for social animals. For example, the ability to discriminate illness behavior would be helpful for avoiding spoiled foods. We report on an experiment with Japanese quails testing whether these birds can discriminate the physical states of conspecifics. The quails were trained to discriminate between moving video images of quails injected with psychoactive drugs and those in a normal (not injected) condition. Methamphetamine (stimulant) or ketamine (anesthetic) were used to produce drug-induced behaviors in conspecifics. The former induced hyperactive behavior and the latter hypoactive behavior. The subject quails could learn the discrimination and showed generalization to novel images of the drug-induced behaviors. They did not, however, show discriminative behavior according to the type and dosage of the drugs. Thus, they categorized the behavior not on the basis of degree of activity, but on the basis of abnormality.

Keywords Discrimination · Drug-induced behavior · Ketamine · Methamphetamine · Quails

Introduction

Both humans and animals have the ability to categorize the behaviors of a conspecific – an ability crucial for iden-

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tifying its intention, predicting its next behavior, and deciding their own behaviors. Discriminative stimulus properties of behavior have been widely observed in field research. Experimentally, Millard (1979) trained pigeons to discriminate among responding patterns of other pigeons in an operant chamber. The subjects could learn to discriminate between a high response rate and a low response rate. In addition, pigeons could discriminate between still images and moving images of a conspecific and maintained the discrimination when other types of motion were presented (Dittrich and Lea 1993). Thus, this finding suggests that pigeons might possess a concept of generalized motion.

Dolphins can be taught complex instructions by gesture (e.g., Herman et al. 1990) and it is widely known that companion animals easily discriminate small actions of their owners. We trained pigeons to discriminate between two words of Japanese sign language displayed on a TV monitor (Watanabe and Furuya 1998). The pigeons could learn to discriminate between the gestural signs, but they did not show transfer of the discrimination when a novel demonstrator performed the sign language. Thus, we hypothesized that discrimination of conspecific behaviors may serve a different function than discrimination of behaviors performed by a member of a different species.

Detection of "abnormal" behavior is especially important because an abnormal conspecific may be dangerous or may be a signal for a dangerous environment. For example, an animal can avoid toxic food by observing illness behaviors in other animals that have consumed toxic food. In fact, social transmission of taste aversion has been observed in red-winged black birds (Mason and Reidinger 1982) although social transmission of taste aversion has not been confirmed in rats (Galef et al. 1983, 1990). In an experimental situation where an electric shock given to a pigeon in a neighboring chamber was a signal (CS) for the delivery of an electric shock to an observer, the shock-induced behavior of the neighbor markedly suppressed the operant behavior of the observer (Watanabe and Ono 1986). This finding suggests that abnormal behaviors have aversive stimulus properties. Humphreys and Einon (1981) examined social preferences of rat pups

using a T-maze. One mate pup was placed at the end of one arm of the maze and another mate was placed at the end of the other arm in a cage. The pups showed preference for the mate who could make contact directly with them. When a mate injected with methamphetamine (2.0 mg/kg) was placed at one arm and a normal mate at the other end, the subjects preferred to enter the arm where the normal mate was placed.

The aim of this study is to examine the ability of Japanese quails to discriminate the physical states, abnormal or normal, of conspecifics. We used Japanese quails as subjects because they are omnivorous and social animals. If the ability to detect abnormal states in others is advantageous, then such an ability would be expected to be well developed in animals such as quails.

Defining abnormal behavior is not easy. However, we defined abnormal behavior as a deviation from normal behavioral patterns in either of two directions: hyperactive or hypoactive. We employed a stimulant (methamphetamine) to induce hyperactive behavior and a depressant (ketamine) to induce slightly paralyzed behavior in quails. One group of quails was taught to discriminate between moving video images of hyperactive quails and normal quails and the other group was taught to discriminate between those of hypoactive quails and normal quails. Then, video clips of quails injected with different doses of drugs were presented to examine generalization of their categorization. We found that their categorization was based on the discrimination between normal and abnormal behavior, rather than between hyperactive or depressive behavior.

Methods

Subjects

Four male, experimentally naive, adult, Japanese quails (*Coturnix coturnix japonica*) (Charles River, Japan) were used. Quails were housed individually and were maintained at about 80% of their free-feeding weights throughout the experimental period. The ambient temperature was maintained at 24°C and water was freely available in the cages. The illumination was controlled in 12L:12D cycles.

Apparatus

An operant chamber (32×31×45.5 cm) designed for quails was used. The front panel had a response key (5×7 cm) made of transparent glass through which the subjects could see a TV monitor (KV-6 PR1, Sony) placed 11 cm behind the key. The distance from the floor to the pecking key was 11 cm and an opening for the feeder was located below the key. The subjects could get food for quails (NQ-1, Nisseiken) from the feeder when it was operated. The TV monitor was connected to a laser disk player (LV-210A, Teac). A personal computer (MSX2+, Sanyo) was used to control the experiment. The chamber was placed in a sound-attenuating wooden cubicle. White noise (75 dB outside the cubicle) was broadcast during the experiment.

Stimuli

The training stimuli were color video images of two male and two female quails under three different conditions: normal, depressant and stimulant. These stimulus quails were different from those trained in the experiment. Under the depressant condition, the quails were injected with ketamine (20 mg/kg; ketalar 50, Sankyo) into the pectoral muscle. Under the stimulant condition, methamphetamine HCl (12 mg/kg) was injected. These quails were placed individually in a transparent glass box (45×60×45 cm) and videotaped by an 8 mm VCR (MB-J10, Canon). Food was scattered on the floor of the box and the birds were able to eat during recording. Portions of the video records that demonstrated typical drug-induced behavior were selected arbitrarily. After injection of ketamine, the quails walked slowly and sometimes tottered, whereas after injection of methamphetamine, they maintained an upright position with an open beak and displayed rapid movement of the throat muscle.[1] Four (one per subject) different video clips from the three conditions, that is, 12 clips in total, each lasting 20 s, were recorded on a laser disk.

In the test sessions, we used the novel video clips of quails injected with ketamine (20 mg/kg) or methamphetamine (12 mg/kg) to examine the generalization of trained physical states to novel video clips. In addition, clips of the quails that were injected with other doses of the drugs (40 mg/kg ketamine or 15 mg/kg methamphetamine) were introduced to determine whether discrimination could be generalized to novel (but in the same direction), abnormal states or not. The higher dose injection of ketamine caused motor deficiency in the quails and sometimes they lay down on the floor, kicked in the air, and then stood up again. The quails' behavior after the injection of 15 mg/kg methamphetamine did not differ much from that after the 12 mg/kg injection, but they moved more rapidly after the injection of the higher dose. A total of 32 novel clips, 8 ketamine (4 from 20 mg/kg, and 4 from 40 mg/kg), 8 methamphetamine (4 from 12 mg/kg, and 4 from 15 mg/kg) and 16 normal quails, were recorded on the laser disk. The luminance in front of the key was 30–38 lx.

Procedure

All subjects were trained to peck the response key. The reinforcement was the presentation of the feeder for 4 s. Then, they were divided into two groups – two for depressant and two for stimulant groups – for discrimination training. In the depressant group, the discriminative stimuli for food reinforcement (S+) were the video images of the ketamine-injected birds, whereas the images of the methamphetamine-injected birds served as the S+ in the stimulant group. Both groups of subjects could not get any food when they were shown video images of normal quails (S–).

Responses were reinforced according to a reinforcement schedule of tandem fixed time (FT) 10 s and variable interval (VI) 4 s when the S+ appeared on the TV monitor. That is, the stimuli were presented for the first 10 s without reinforcement, then the responses were reinforced under VI 4 s. The stimulus was turned off when the reinforcement was given and a 5-s intertrial interval followed. The stimulus was displayed for a maximum of 20 s. When the subjects failed to respond during this period, the stimulus was extinguished and the intertrial interval started. No stimuli were displayed during the intertrial interval. When S– appeared on the TV monitor, responses were never reinforced; other conditions were identical to those for the S+ period. One training session consisted of 40 trials, with 4 presentations (in Gellerman series) of 4 different records of S+ and 4 different records of S–. We used the index of rho (ρ) to evaluate the discrimination (cf. Herrnstein et al. 1976). To calculate the value of ρ, the number of responses emitted during the FT 10 s was used to avoid uneven chance responses during the VI phase. Training continued until the subjects showed a ρ value higher than 0.8 for two consecutive sessions.

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[1] We decided to use ketamine and methamphetamine as depressant and stimulant drugs because they are comparatively safer than any other drugs that can induce similar types of behavior. The doses used were the minimum doses at which we could observe some typical drug-induced behaviors. The quails recovered from the injections within less than 2 h and showed no observable aftereffects of the drugs.

After the subjects reached the criterion of discrimination, they were given a generalization test. A test session consisted of 32 trials, each using a different stimulus selected from 16 novel records of normal quails, 4 records each of quails injected with 20 mg/kg and 40 mg/kg ketamine, and 4 records each of quails injected with 12 mg/kg and 15 mg/kg methamphetamine. These stimuli appeared in a random order. The stimuli were displayed in the same manner as in the training session except that no reinforcement was given during the test. The subjects were tested twice and 2 training sessions were inserted between the tests to maintain their discriminative behavior. The stimulus sequence was varied between the two tests.

Results

Figure 1 shows the learning curve of each subject during the discrimination training. To reach the acquisition criterion of over a 0.8 ρ value, the subjects in the depressant group took 27 and 55 sessions (41 on average), whereas those in stimulant group took 24 and 31 sessions (27.5 on average). There was no significant difference between the number of sessions in the groups [$t(2)=0.93$, $P=45$].

To examine discrimination ability among the conditions of drug-injected behavior and normal behavior, we calculated the average number of responses during the stimulus presentation for each of the three stimulus categories (ketamine- or methamphetamine-injected and normal controls). Figure 2 (left panel) shows the mean number of responses per test trial of the depressant group. As shown, there was a significant difference among the stimulus conditions (one-way ANOVA, $F(2,125)=5.34$, $P=0.006$). A posteriori multiple comparison test (Bonferroni method) revealed that the difference between the mean responses to the video clips of methamphetamine-injected quails and normal quails was significant [$t(125)=3.78$, $P=0.00003$]. Fig-


Fig. 1 Rho (ρ) values during discrimination acquisition for each subject in the depressant group (*top panels*) and in the stimulant group (*bottom panels*). *Upper* and *lower bars* in each *panel* indicate a ρ value of 0.8 (criterion performance) and chance level performance of 0.5, respectively
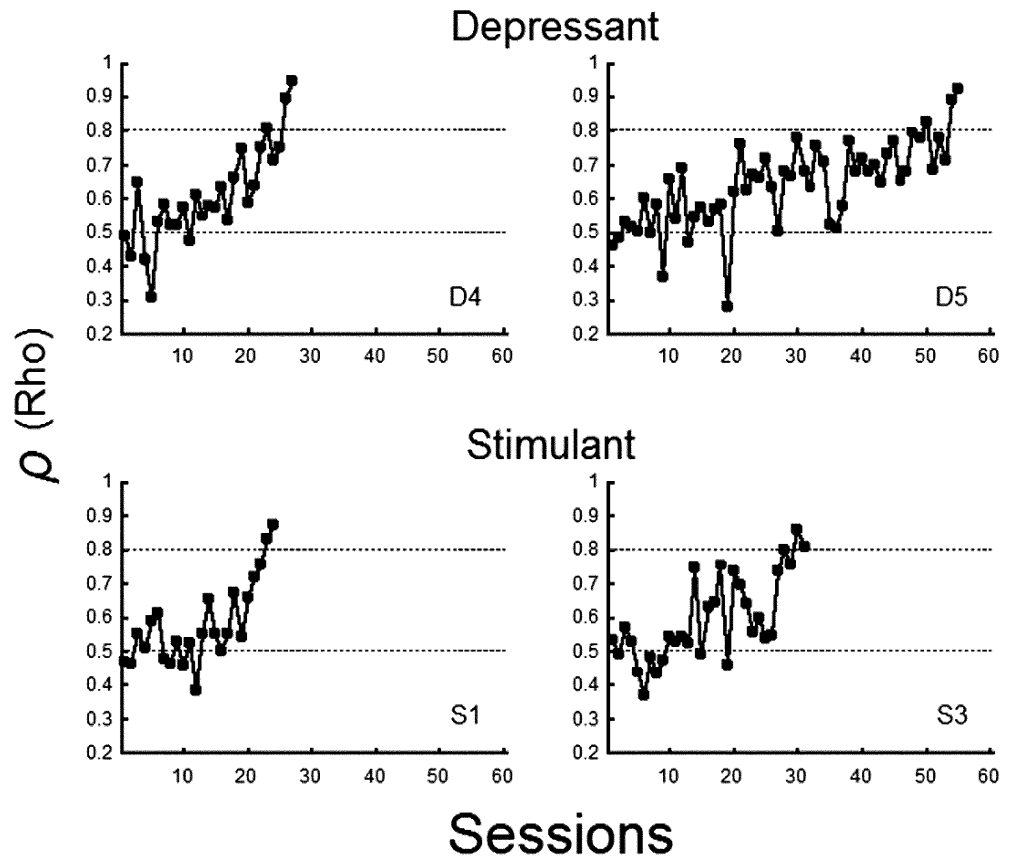
Fig. 2 Mean number of responses per test trial of the depressant group (*left panel*) and of the stimulant group (*right panel*) across the generalization tests in experiment 1. Each *bar* corresponds to a specific condition of video images (ketamine-injected, methamphetamine-injected and normal quails)
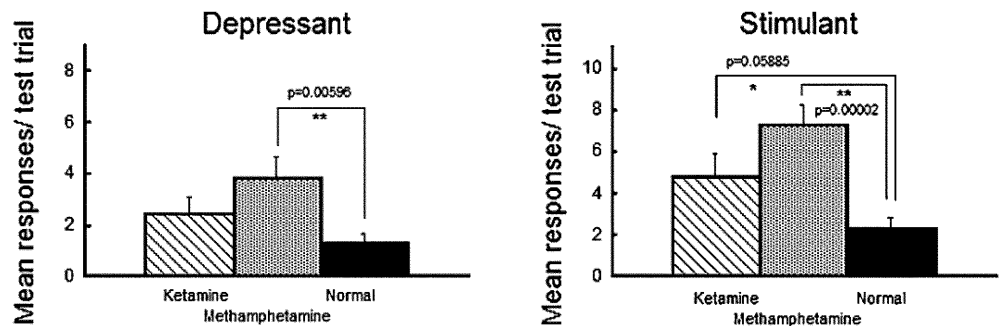
Fig. 3 Rank of the number of responses made by each quail subject in the depressant group (*n*=2, *top panel*) and in the stimulant group (*n*=2, *bottom panel*) in the generalization tests. *M12* indicates images of methamphetamine-injected quails (12 mg/kg); *M15* indicates those of methamphetamine-injected quails (15 mg/kg); *K20* indicates those of ketamine-injected quails (20 mg/kg); *K40* indicates those of ketamine-injected quails (40 mg/kg); and *NORMAL* indicates those of the quails in the normal condition (not injected)
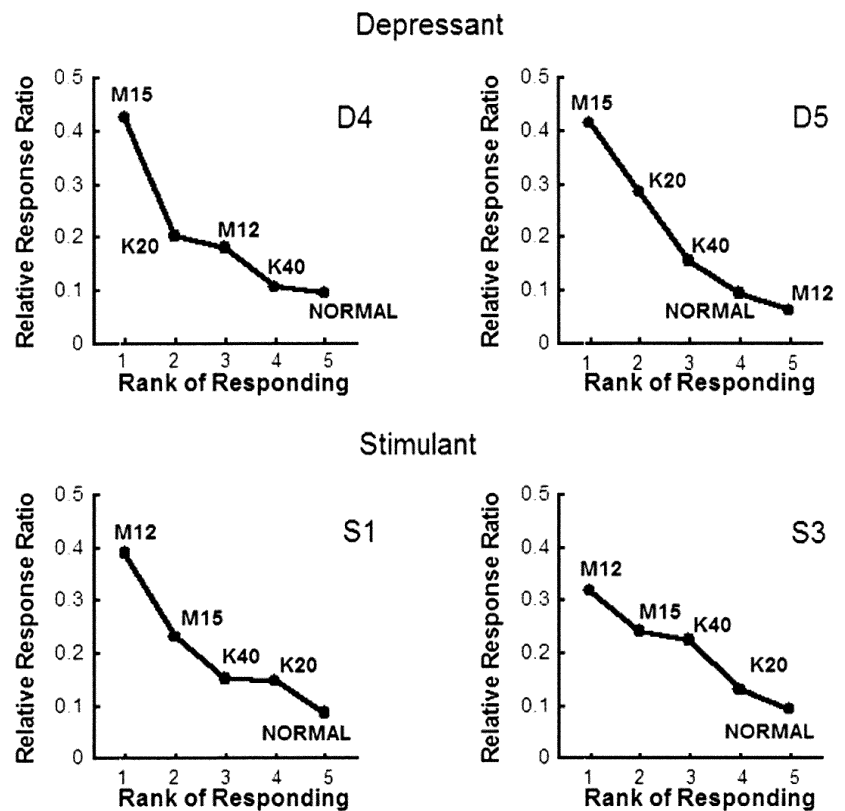
ure 3 shows the rank of the relative response ratios of the generalization test for each subject. The relative response ratio is defined as the ratio of the responses to a given category, relative to the total number of responses in all the generalization tests. The results of the two tests were averaged for each subject. In the depressant group, both birds emitted responses more often to the images of the 15 mg/kg methamphetamine-injected birds (not the S+ condition) than to the images of the 20 mg/kg ketamine-injected birds (S+ condition). The ρ value between responses to the abnormal images (both ketamine- and methamphetamine-injected quails) and the normal images was 0.62 for quail D4 and 0.65 for quail D5. These response patterns imply that the quails generalized discrimination from the trained images (20 mg/kg ketamine) to the novel images of quails injected with the same dose. Spearman's rank order correlation between the two birds was r_s=0.7 (*n*=5, *P*=0.19).

The right panel of Fig. 2 shows the averaged number of responses of the stimulant group for each category of the stimuli presented in the test sessions. There was a significant difference among stimulus conditions [one-way ANOVA, $F_{(2,125)}$=11.33, *P*=0.00003]. A multiple comparison test (Bonferroni method) revealed that the difference between the response ratios to the images of methamphetamine-injected quails and normal quails was significant [$t_{(125)}$=7.25, *P*<0.001]. The quails showed more responses to the images of ketamine-injected quails than to those of normal quails, although the difference was not significant [$t_{(125)}$=4.81, *P*= 0.05885]. As shown in Fig. 3,

quail 1 responded most frequently to the 12 mg/kg methamphetamine condition (S+ condition), while quail 3 pecked the same stimuli less frequently than any other stimuli that had been drug-injected. The ρ value between the responses to the abnormal and the normal quails was 0.72 for quail 1 and 0.73 for quail 3. Both birds responded least frequently to the normal stimuli, suggesting that the subjects showed discrimination between the normal and the drug-induced behaviors. The correlation between the subjects was r_s=0.4 (*n*=5, *P*=0.50).

Discussion

The present results clearly demonstrate that quails can discriminate moving video images of conspecific behaviors. They could learn discrimination between the normal birds and drug-injected birds and showed generalization to the novel images of the drug-injected birds.

The response triggered by moving video images differs among different species. Cichlid fish show breeding behavior to video images of conspecifics (Balshine-Earn and Lotem 1998); jumping spiders attack televised prey and try to court televised conspecifics (Clark and Uetz 1990); jungle fowls behave differentially to video images of conspecifics standing near a food dish and to those actually feeding (Mcquoid and Galef 1993); chickadees emit gargle vocalizations to video images (Baker et al. 1996); and pigeons show courtship displays in response to video-taped images of conspecifics (Shimizu 1998). Pigeons can

also discriminate among categories of conspecific movement, such as pecking or walking (Dittrich et al. 1998). On the other hand, life-size video images of conspecifics do not affect the feeding behavior of hens (D'Eath and Dawkins 1996). Hens learn individual discrimination using video images quite slowly while they rapidly learn the discrimination with real birds (Patterson-Kane et al. 1997). Pigeons do not show any social responses to moving video images of conspecifics (Ryan and Lea 1994). Factors such as species differences in critical fusion frequency (CFF) or the type of behavior tested (i.e., feeding, courtship or discrimination) may have caused these differences. Because CFF depends on the luminance of the screen, flickering may have affected the results in some experiments (cf. D'Eath 1998; Lea and Dittrich 2000).

The quails in the present experiment did not show systematic discrimination according to the type or dose of the drugs. As mentioned in the introduction, pigeons also seem to classify different types of motions into one class, "motion" (Dittrich and Lea 1993). The present results support such types of categorization, because they classified the hyperactive behavior induced by methamphetamine and hypoactive behavior induced by ketamine in a category of abnormal behavior. In other words, the quails did not classify different behaviors of conspecifics along the "activity" dimension, but classified different drug-induced behaviors in the same category that was different from that of normal behavior.

The quails of both groups showed more responses to the images of methamphetamine-injected quails than to those of ketamine-injected quails. It was not clear whether the images of methamphetamine-injected quails is easier to discriminate than those of ketamine or not. However, we conducted a similar experiment with human subjects using the same stimuli as in the present study, and their verbal reports suggested this possibility (Yamazaki et al., unpublished data). That is, they found that the methamphetamine-injected quails had more salient cues than the ketamine-injected quails, such as small movement of the throat and walking around quickly.

In summary, the present experiment showed successful discrimination between two types of conspecific behaviors, normal and abnormal, and this ability could be generalized even to novel stimuli. Of course, whether the quails actually use this kind of social observation, for example, to avoid toxic food remains unclear. The results that the quails categorize on the basis of abnormality, not on the basis of specific condition, however, may suggest that they process information from other conspecifics adaptively to survive in the wild.

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