Short communications to Journal of Comparative Psychology

Japanese monkeys (*Macaca fuscata*) spontaneously associate alarm calls with snakes appearing in the left visual field

Abstract

Many socially living animals are sensitive to a potential predator as part of their anti-predator strategy. Alarm calls function to deter predators and to help other group members detect danger. The left visual field is involved in detection of potential threats or predators in many vertebrates, but it is unclear how alarm calls influence visual detection of a potential predator. Here, we experimentally examined how alarm calls spontaneously influence the search for pictures of a potential predator in captive Japanese macaques. We used an audiovisual preferential-looking paradigm by presenting pictures of a snake and a flower simultaneous with either a recording of alarm calls or contact calls. We found no difference in gaze duration between the two picture types when playing back contact calls. Monkeys looked significantly longer at pictures of snakes than at those of flowers when alarm calls were played back if the snake pictures were presented on the left side of the monkey's visual field, indicating right hemispheric bias during processing of predator representations. This is the first laboratory demonstration of auditory enhancement of visual detection of predators in the left visual field in animals, which will contribute to a better understanding of alarm call studies conducted in the wild.

Keywords: anti-predator strategy; visual lateralization; alarm call; snake fear; nonhuman primates

Human and nonhuman primates appear extremely sensitive to biologically threatening stimuli such as snakes (LoBue, Rakison, & DeLoache, 2010). Humans are easily conditioned to react to pictures of snakes, and this conditioned fear of snakes is resistant to extinction (see Ohman & Mineka, 2001, 2003 for a review). Similar to humans, laboratory-reared rhesus monkeys are quickly conditioned to fear snakes after watching videotapes of monkeys being frightened by snakes (Cook & Mineka, 1990). Humans and nonhuman primates also detect snakes faster than they detect neutral stimuli (e.g., flowers) during visual search tasks (LoBue et al., 2010). Even young children and lab-reared monkeys with no experience with snakes are faster to detect snakes than flowers (LoBue & DeLoache, 2008; Shibasaki & Kawai, 2009).

Visual lateralization has been revealed during visual processing of threatening stimuli in primates and many other vertebrates (see Rogers & Andrew, 2002; Rogers, Vallortigara, & Andrew, 2013 for reviews). Horses react more strongly to a frightening stimulus when it is presented in the left visual field (Austin & Rogers, 2007), and horses use the left eye to view a potential threat or predator (Farmer, Krueger, & Byrne, 2010). The stripe-faced dunnart showed the higher reactivity when the subjects were presented with a model snake on their left side (Lippolis, Westman, McAllan, & Rogers, 2005), thus indicating specialization of the right side of the brain in controlling the escape response, as found previously in toads (Lippolis, Bisazza, Rogers, & Vallortigara, 2002). Gelada baboons direct more aggressive responses to conspecifics on their left side than they do to those on their right side (Casperd & Dunbar, 1996). The left visual field bias likely reflects right hemisphere dominance during visual processing of emotional information (Rogers et al., 2013).

Similar to the visual system, specialized cognitive processing has evolved during auditory communication in many animals. Many socially living animals produce predator-specific alarm calls as part of their anti-predator strategy (Zuberbuhler, 2003 for review). Alarm calls convey representation of potential predators and further evoke animal escapes. Despite clear evidences for association of predator presence and alarm calls, it has been unclear how alarm calls influence detections of potential predators. For the learning of alarm calls, experiences like observing demonstrators would be necessary for nonhuman primates (e.g., Campbell & Snowdon, 2009).

The aim of this study was to investigate how alarm calls spontaneously influence detection of potential predator pictures by captive Japanese macaques, particularly if they are presented in the left visual field. We assessed this with an audiovisual preferential-looking paradigm in which Japanese macaques were simultaneously presented with alarm calls and pictures of a predator. Audiovisual preferential-looking paradigms have been used to investigate matching ability in the auditory and visual sense modalities in animals (Ghazanfar & Logothetis, 2003) and human infants (Kuhl & Meltzoff, 1982). In a typical task, pairs of images (or movies) are presented to subjects in conjunction with an auditory stimulus. The rationale is that if the auditory stimulus has the power to evoke recall of an associated mental representation or emotion, then subjects are expected to look longer at the matching visual stimulus relative to a control stimulus.

In our experiments, we paired pictures of snakes and flowers with the Japanese macaques' alarm and contact calls. If alarm calls emotionally enhance visual detection of a potential predator, biased visual detection of threatening stimuli would occur more clearly.

Method

Subjects. We used 16 female Japanese macaques (*Macaca fuscata*) aged 2–6 years. All were born into social groups housed in outdoor enclosures at the Primate Research Institute, Kyoto University and naïve to seeing snakes.

Apparatus. Subjects were tested in a custom-made experimental box (450 mm W × 450 D ×

600 H) that was positioned in a sound attenuating chamber (RE-246, TRACOUSTICS). Three sides of the experimental box were covered with transparent polycarbonate boards, and the other was the cage gate. Subjects were tested individually in the experimental box using a 22-inch LCD screen (ProLite E2208HDS, IIYAMA, Tokyo, Japan) mounted on the experimental box, which enabled subjects to look at the monitor. The screen was connected to a computer placed outside the sound chamber for stimulus presentation. The screen resolution was set to 1600 × 800 pixels. A small 8.5-mm 1-in -pinhole infrared-sensitive CCD camera (40 × 25 × 36 mm, ITC-401, ITC, Tokyo, Japan) placed at the center of the monitor was connected to a TV screen (LC-22K5, SHARP, Japan) outside the sound chamber for gaze analysis and to a video camera (GZ-MG840-A, Victor, Japan) to record the subject's behavior during experiments. An active speaker (SRS-Z100, Sony, Tokyo, Japan) placed under the center of the LCD screen was used to deliver the playback stimuli.

Visual Stimuli. We used two snake and two flower pictures as visual stimuli. The pictures, which were available from the internet (http://www.pitt.edu/~mcs2/herp/SoNA.html, http://www.flowerpictures.net/flower_pictures.htm), and used in previous studies (Shibasaki & Kawai, 2009). The pictures were reformatted to 300 × 400 pixels. The snakes used for stimuli were species which wild Japanese macaques never naturally see in the forest in order to neglect the possible effect of "innate" reactions to specific snake species which wild macaques can see. The average luminance and contrast were equalized across all stimuli with Adobe Photoshop CS5.

Auditory Stimuli. We used an alarm call and a contact call series as auditory stimuli (see online supplementary Figure S1). Both calls were recorded from free-ranging Japanese macaques by HK in 2006. Alarm calls of Japanese macaques were defined as being of only a single type given to dogs, snakes and other potential predators (Green 1975). Contact calls are frequently exchanged among group members during the movement to maintain their cohesion (Koda, 2004). Both playback stimuli consisted of a series of three calls delivered at the same rate over a total duration of 10 s. Calls were edited to match the maximum intensities between calls using Adobe Audition 2.0.

Procedure. Prior to each trial, we displayed a fixation cross in the center of the screen to draw the monkeys' attention. Once this occurred, the experimenter simultaneously displayed pictures of a snake and a flower on the screen. The distance between the left and right image centers was 394.9 mm. Presentation time was 10 s. We simultaneously broadcasted one auditory stimulus as a 10-s series consisting of either three alarm or three contact calls. The trial was performed once in a day for each subject. Two trials per subject were performed for each call, counterbalancing the side positions of paired stimuli. Consequently four trials were performed for each subject monkey.

Video Analysis. We measured the total looking duration for both images during the 10-s trial. We categorized subjects' gaze directions as 1) looking to the left side of the screen, 2) looking to the right side of the screen, and 3) not looking at the screen. Coding was carried out by an observer who was unfamiliar with the aims of this study. Coding units consisted of 33-ms-long video frames extracted with custom-made software. The procedure was the same as with our previous study using visual pared comparison tasks (Sato, Koda, Lemasson, Nagumo, & Masataka, 2012)

Statistical Analysis. We performed a three-way repeated-measures analysis of variance (ANOVA) using Statistica 6.1 (StatSoft Japan, Inc. Tokyo, Japan) to examine the effects of call type (alarm vs. contact call), stimulus type (snake vs. flower), and position (left vs. right). If interaction effects were found, we also performed post-hoc pairwise comparisons using Tukey's honestly significant difference test. Significance levels were all set to $p < .05$.

Ethical Note. All procedures were approved by the ethics committee of Primate Research Institute of Kyoto University (permit number 2011-070) and were in accordance with the Guide for the Care and Use of Laboratory Primates (Third Edition of Primate Research Institute, Kyoto

University).

Results

Prior to analysis, we performed Levene test to check the normal distribution of the data, and confirmed that our data were normally distributed, $p > 0.13$. The repeated-measures ANOVA revealed a significant main effect of stimulus type, F (1, 30) = 10.13, $p < .005$, $\eta_p^2 = .25$, but not of call type, F (1, 30) = 1.01, $p = .32$, $\eta_p^2 = .03$, and position, F (1, 30) = 3.14, $p = .09$, $\eta_p^2 = .09$. More importantly, a three-way interaction effect was found in looking time (LT), F (1, 30) =4.70, $p < .05$, $\eta_p^2 = .14$. To examine how three main effects interacted with each other, post-hoc comparisons were performed. The post-hoc analyses further revealed that this was due to a significant difference in looking time between the snake and flower pictures in the alarm condition (mean LT ± 95% confidence interval, snake: 52.50 ± 15.37 frames; flower: 21.44 ± 10.71 frames, $p = .02$, Figure 1), but not in the contact-call condition (snake: 39.50 ± 16.93 frames; flower: 23.69 ± 8.93 frames, $p = .65$, Figure 1), when snake pictures were presented on the left, indicating that monkeys looked significantly longer at pictures of snakes when they heard alarm calls and the snake picture was presented in the left visual field. In contrast with the condition where the snake picture appears on the left side, there is no significant difference in looking time between the snake and flower pictures in both the alarm condition (snake: 37.75 ± 12.44 frames; flower: 40.81 ± 12.32 frames, $p = .99$, Figure 1) and the contact-call condition (snake: 64.69 ± 20.36 frames; flower: 44.69 ± 14.50 frames, $p = .36$, Figure 1), when snake picture was presented on the right side.

Discussion

Our results are the first laboratory-based demonstration in nonhuman animals that predator alarm calls enhance looking at predator images. Subjects looked longer at pictures of snakes when they heard alarm calls compared with when they heard contact calls, suggesting specialized cognitive traits for audio-visual processing of fear related stimuli. This would agree with the notion that the amygdala responds audio-visually to fear related stimuli (Kuraoka & Nakamura, 2007).Furthermore, our study showed left-side bias during snake picture processing under the alarm call condition, suggesting right-hemispheric neural bias. Right-hemisphere dominance in amygdala processing has also been found in humans during processing of fear-relevant stimuli such as angry human faces (Gainotti, 2012). Equally relevant studies have found a left-eye bias in response to frightening stimuli in vertebrates, including fish, frogs, lizards, birds, rodents, dogs, horses, and primates (Kaplan & Rogers, 2013; Vallortigara & Rogers, 2005 for review). Particularly, recent studies in dogs have revealed the biased monitoring reactions to social stimuli. The experiment using a head-orienting procedure showed that dogs turned their head to the right side (left hemisphere) in response to conspecific vocalizations, but to the left side in response to the sound of the thunderstorm (Siniscalchi, Quaranta, & Rogers, 2008). Dogs turned preferentially their head to the left side in response to the silhouettes of snakes (Siniscalchi, Sasso, Pepe, Vallortigara, & Quaranta, 2010). The two studies suggests dog's biased processing in right hemisphere for audio-visual fear related stimuli. The similar right hemisphere dominance has been found in primates. The common marmosets displayed right-eye preferences for viewing a piece of preferred food (Hook-Costigan & Rogers, 1998). However, when the marmosets viewed a model snake, they displayed increased arousal and the eye preferences shifted away from a preference for the right eye to a left-eye preference (Hook-Costigan & Rogers, 1998). Our findings are in line with these previous findings, suggesting a hemispheric bias in the neural mechanisms of emotional processing, particularly fear processing.

Snake fear in humans and nonhuman primates is often discussed in terms of underlying learning processes. Pioneer studies in macaques and squirrel monkeys have found that fear of snakes is selectively and rapidly learned, suggesting a biological preparedness for conditioning of snake fear (Mineka, Davidson, Cook, & Keir, 1984). According to this “preparedness theory” (Seligman, 1971), some natural stimuli are easier to condition to fearful responses than others, suggesting that animals are evolutionarily prepared to learn some associations more rapidly and effectively than others. Experiments with laboratory-reared monkeys naive to snakes have shown that they rapidly acquire a fear of snakes by observing other monkeys showing fear responses to snakes (Mineka & Öhman, 2002). Such learning is strong and persistent (Cook, Mineka, Wolkenstein, & Laitsch, 1985; Mineka et al., 1984), even if the fearful model monkey is shown on video (Cook & Mineka, 1990). Of particular importance is that our subjects reared in captivity were naive to snakes. In conclusion, monkeys appear to have an innate sensitivity to snakes, and alarm calls appear to be particularly suited to this process.

We showed spontaneous lateralized looking at a predator evoked by alarm calls in monkeys, suggesting audiovisual correspondence during processing of emotional stimuli, demonstrated by the association between predator images and alarm calls. Here we hypothesize that learning a fear of snakes requires experience, whereas the association between alarm calls and potential predators is innate. However, we should acknowledge some research limitations of limited set of stimuli used here. The further study with a large number of stimulus sets would be necessary to generalize our findings. Furthermore, we need to compare the snake pictures to other pictures instead of flowers and replicate our findings to reject the possibility that the results might just be based on the lack of preference for snakes when presented on the right side with alarm call.

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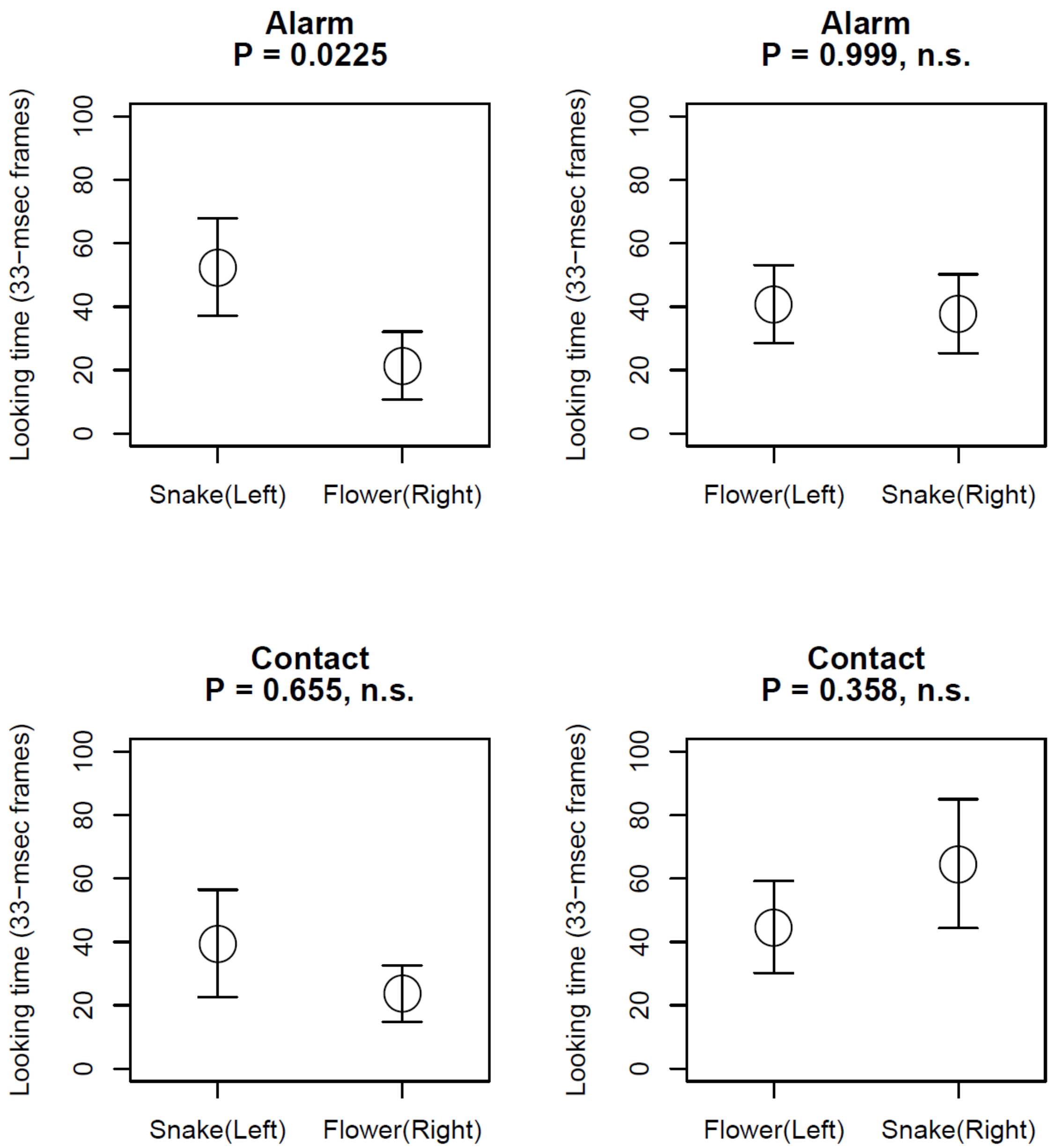
Figure legend

Figure 1. Looking duration of Japanese macaques at images of snakes and flowers presented to the left or right visual field while hearing recordings of conspecific alarm calls or contact calls (means ±95% confidence intervals)

Online Supplementary Figure legend

Figure S1. Sound spectrogram of calls used for stimuli in the experiments.

Figure 1


Alarm
P = 0.0225
Looking time (33−msec frames)
0
20
40
60
80
100
Snake(Left)
Flower(Right)
Alarm
P = 0.999, n.s.
Looking time (33−msec frames)
0
20
40
60
80
100
Flower(Left)
Snake(Right)
Contact
P = 0.655, n.s.
Looking time (33−msec frames)
0
20
40
60
80
100
Snake(Left)
Flower(Right)
Contact
P = 0.358, n.s.
Looking time (33−msec frames)
0
20
40
60
80
100
Flower(Left)
Snake(Right)

Online supplementary material

Figure S1