**Neurophysiological study for pulvinar role in rapid detection of snakes in monkeys**

生命融合科学教育部

システム情動科学

Le Van Quan

**Abstract**

Snakes and their relationships with humans and other primates have attracted broad attention from multiple fields of study, but not, surprisingly, from neuroscience despite the involvement of the visual system and strong behavioral and physiological evidence that humans and other primates can detect snakes faster than innocuous objects. Here, we report the existence of neurons in the primate medial and dorsolateral pulvinar that respond selectively to visual images of snakes. Compared to three other categories of stimuli (monkey faces, monkey hands, and geometrical shapes), snakes elicited the strongest, fastest responses, and the responses were not reduced by low spatial filtering. These findings integrate neuroscience with evolutionary biology, anthropology, psychology, herpetology, and primatology by identifying a neurobiological basis for primates' heightened visual sensitivity to snakes, and adding a crucial component to the growing evolutionary perspective that snakes have long shaped our primate lineage.

Keywords: Key words; evolution, primates, Snake Detection Theory, pulvinar, visual responses

## Introduction

Snakes have long been of interest to us above and beyond the attention we give to other wild animals. Their attributes and our relationships with them have been topics of discussion in fields as disparate as religion, philosophy, anthropology, psychology, primatology, and herpetology (Isbell, 2009; Headland and Greene, 2011). Ochre and eggshells dated to as early as 75,000 years ago and found with cross-hatched and ladder-shaped lines (Henshilwood et al., 2002, Texier et al., 2010) resemble the dorsal and ventral scale patterns of snakes. As the only natural objects with those characteristics, snakes may have been among the first models used in representational imagery created by modern humans. Our interest in snakes may have originated much farther back in time; our primate lineage has had a long and complex evolutionary history with snakes as competitors, predators, and prey (Headland and Greene, 2011). The position of primates as prey of snakes has, in fact, been argued to have constituted strong selection favoring the evolution of the ability to detect snakes quickly as a means of avoiding them, beginning with the earliest primates (Isbell, 2006, Isbell, 2009). Across primate species, ages, and (human) cultures, snakes are indeed detected visually more quickly than innocuous stimuli, even in cluttered scenes (Öhman et al., 2001; Shibashaki and Kawai, 2009; LoBue and DeLoache, 2010; Masataka et al., 2010; Soares, 2012; Penkunas and Coss, 2013). Physiological responses reveal that humans are also able to detect snakes visually even before becoming consciously aware of them (Öhman and Soares, 1993). Although the visual system must be involved in the preferential ability to detect snakes rapidly and pre-consciously or automatically, the neurological basis for it has not yet been elucidated, perhaps because an evolutionary perspective is rarely incorporated in neuroscientific studies. Our study helps to fill this interdisciplinary gap by investigating the responses of neurons to snakes and other natural stimuli that may have acted as selective pressures on primates in the past.

Here, we identify a mechanism for the visual system's involvement in rapid snake detection by measuring neuronal responses in the medial and dorsolateral pulvinar to images of snakes, faces of monkeys, hands of monkeys, and geometric shapes in a catarrhine primate, *Macaca fuscata.* The medial and dorsal part of the traditionally delimited lateral pulvinar are distinctive in primates, with no homologous structures found in the visual systems of nonprimate mammals (Preuss, 2007), and the medial pulvinar appears to be involved in visual attention and fast processing of threatening images (Ward et al., 2005). Based on this and other indirect evidence, the Snake Detection Theory (Isbell, 2009) hypothesized that these

primate-specific regions of the pulvinar evolved in part to assist primates in detecting and thus avoiding snakes. If true, then we would expect snake-sensitive neurons to be found in those regions. Here we present the first neuroscientific evidence in support of the Snake Detection Theory (Isbell, 2009).

## Materials and Methods

### Animals

Two adult (1 female and 1 male) macaque monkeys (*Macaca fuscata*), weighing 7.0-8.8 kg, were used in this experiment. The monkeys were born and kept in a walled-off enclosure at a national monkey farm in Amami Island in Japan for two yrs, and then kept inside at the University of Toyama, Japan for the experiment. We believe that they had no chance to encounter snakes before the experiment. Each monkey was individually housed with food available ad libitum. The monkeys were deprived of water in their home cage and received juice as a reward during training and recording sessions. Supplemental water and vegetables were given after each day's session. To assess the monkeys' health, their weight was routinely monitored. The monkeys were treated in strict compliance with the United States Public Health Service Policy on Human Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Guidelines for the Care and Use of Laboratory Animals of the University of Toyama. This study has been approved by the Committee for Animal Experiments and Ethics at the University of Toyama. The monkey sat in a monkey chair 68 cm away from the center of a 19-inch computer display for behavioral tasks during the training and recording sessions in a shielded room. The CRT monitor was set so that its center was on the same horizontal plane as the monkey's eyes. The monkey chair was equipped with a responding button, which was positioned so that the monkey could easily manipulate it. An infrared charge-coupled device (CCD) camera for eyemovement monitoring was firmly attached to the chair by a steel rod. During training and recording sessions, the monkey's eye position was monitored with 33 ms time resolution by an eye-monitoring system (Matsuda, 1996). The juice reward was accessible to the monkey through a small spout controlled by an electromagnetic valve. A visual stimulus generator (ViSaGe MKII Visual Stimulus Generator, Cambridge Research Systems, UK) controlled the electromagnetic valve, the timing of visual stimuli onset.

**Visual stimuli**

Figure 1A shows the stimulus set, consisting of consisting of photos of snakes, photos of monkey faces (angry and neutral faces), photos of monkey hands and simple geometrical figures (circle, cross, square and star) used in the present study. The species of snakes used in the study were a Cottonmouth (*Agkistrodon piscivorus*) (sn1), a Tsushima Island pitviper (*Gloydius tsushimaensis*) (sn2, 3) and a Japanese mamushi (*Gloydius blomhoffii*) (sn4). We used color images in the present study because previous studies reported that color of the stimuli affected detection of snakes (LoBue and DeLoache, 2008; Hayakawa et al., 2011). The stimuli were 256 digitized RGB color-scale images with their resolution of 270x270 pixels. Stimuli were presented on a black background of 0.7cd/$m^2$ with their centers at the center of the display. The luminance of each stimulus was determined by measuring luminance of the circular area (radius, 6.35 cm) including each stimulus inside the circle by means of a luminance meter (BM-7A; Topcon, Tokyo). The luminance of these color stimuli was almost identical (6.005-6.445 cd/$m^2$) [luminous intensity (total luminance) ranged from 38.432 to 41.248 mcd]. Luminance of the white areas inside the simple geometric patterns was 36.5 cd/$m^2$ (total luminance of the circle, cross, square, and star was 45.6, 38.72, 53.592, and 20.64 mcd, respectively). These stimuli were displayed on a CRT monitor with a resolution of 640 × 480 pixels, and the size of the stimulus area was 5-7 × 5-7°.

**Transformation of visual stimuli**

To analyze what features of the stimuli the neurons responded to, the visual stimuli were transformed. In scrambling, original images were cut into 64 pieces (8 x 8 pieces), and the fragments were randomly reassembled. Figure S3Bb showed a scrambled image of the original snake image (Fig. 1Ba). In spatial filtering, we chose low pass filter (LPF) with 6 cycles/image and high pass filter (HPF) with 20 cycles/image based on previous studies (Vuileumier et al., 2003; Rotshtein et al., 2007). First, colors of each image were separated into 3 color channels (red, green and blue), and converted to grayscale images so that both LPF and HPF could be rendered in grayscale. Then, these three channel images were converted into frequency domain by the Fourier transform. Then, these images in each channel were processed with Gaussian LPF and HPF. Finally, these images in 3 channels were merged (see Fig. 2 for HPF). Figure 1Bc and Bd show the images processed with LPF and HPF, respectively. These images were processed using MatLab 7.0.

**Behavioral tasks**

The monkeys were trained to perform a sequential delayed nonmatching-to sample task (DNMS) that required the discrimination of the visual stimuli (Fig. 1A and B). As illustrated in Fig. 1C, the task was initiated by a buzzer tone. Then, a fixation cross appeared in the center of the display. When the monkeys fixated on the cross for 1.5 s within 0.5-1.0° window, a sample stimulus was presented for 500 ms (sample phase). The control phase was defined as the 100-ms period before the sample phase. Then, after an interval of 1.5 s, the same stimulus appeared again for 500 ms between 1 and 4 times (selected randomly for each trial). Finally, a new stimulus was presented (target phase). When the target appeared, the monkey was required to press a button within 2 s in order to receive a juice reward (0.8 mL). When the monkey failed to respond correctly during the target phase or to press the button before the target phase, the trials were aborted and a 620-Hz buzzer tone was presented. The intertrial intervals (ITI) lasted 15–25 s (Fig. 1C). Visual stimuli were presented in separate blocks; stimulus pairs in the DNMS task within the same block consisted of the same category of stimuli (snakes, faces, hands, and simple geometric patterns). The presentation sequence of each category of the stimuli and presentation sequence of each stimulus within the same category were pseudo-randomly determined so that presentation number of times of each stimulus was equal. After completion of behavioral training, a head-restraining device was attached to the skull under anesthesia (Nishijo et al., 1988; Tazumi et al., 2010). Upon recovery from the surgery, the monkeys were re-trained in the DNMS task while the head was painlessly fixed to the stereotaxic apparatus with the head-restraining device.

**Stereotaxic localization of the pulvinar for recording**

Before recording from the pulvinar in each hemisphere, a tungsten marker (diameter: 500 μm) was inserted near the target area under anesthesia, and three-dimensional magnetic resonance imaging (3-D MRI) scans of the monkey head were performed. The 3-D pictures of the monkey brain with the marker were reconstructed by computer rendering. Three-dimensional stereotaxic coordinates of the target area were determined in reference to the marker in the 3-D reconstructed brain (Asahi et al., 2003). The locations of pulvinar neurons were based on the zero coordinates defined in the stereotaxic atlas of the brain of Macaca fuscata individuals (Kusama and Mabuchi, 1970).

**Recording and analysis of pulvinar neurons**

Neuronal activity was recorded from each hemisphere in both subjects by stereotaxically inserting a glass-insulated tungsten microelectrode into the pulvinar. Spike sorting was performed with the off-line sorter program for cluster analysis (Off-line sorter, Plexon Inc.). Each cluster was checked manually in order to ensure that the cluster boundaries were well separated and that the waveform shapes were consistent with the action potentials. For each isolated cluster, an autocorrelogram was constructed, and only units with refractory periods greater than 1.2 ms were used for further analyses. Finally, superimposed waveforms of the isolated units were drawn in order to check the consistency of the waveforms. Furthermore, all pulvinar neurons were analyzed by autocorrelograms. The autocorrelograms indicated that the refractory periods of the all pulvinar neurons were greater than 2 ms throughout the recording sessions, which indicates that the isolated spikes were recorded from single neurons. We analyzed single neuronal activity during the following 2 periods: 100 ms before (pre) and 500 ms after (post) the onset of stimulus presentation in the sample phase. The baseline firing-rate was defined as the mean firing rate during the 100-ms pre period. Significant excitatory or inhibitory responses to each stimulus were defined by a Wilcoxon signed-rank (WSR) test ($p < 0.05$ for statistical significance) of the neuronal activity between the 100-ms pre and the 500-ms post periods. Furthermore, in order to investigate the temporal changes in the neuronal responses, the 500-ms post period was divided into ten 50-ms epochs. The mean neuronal firing rate was calculated for each of these epochs. The response magnitude was defined as follows: the mean firing rate in each epoch minus the mean firing rate during the 100-ms pre-period.

For each neuron, the response magnitudes during the visual stimulation period (for the whole 500-ms period and for each epoch) for all visual stimuli were analyzed by one-way ANOVA ($p < 0.05$). Response magnitudes between the stimuli were compared by Tukey posthoc tests ($p < 0.05$).

In addition, we analyzed the response latency to each visual stimulus. For each neuron, 1 perievent histogram was constructed with the entire set of data for all trials and all stimuli. Neuronal response latency was defined as the interval from the onset of stimulus presentation to the time at which the neuronal firing rate exceeded the mean ± 2 SD of the baseline firing-rate. All data were expressed as mean ± SEM.

**Multidimensional Scaling analysis (MDS)**

Multidimensional scaling (MDS) is a method that is used to simplify the analysis of

relationships that exist within a complex array of data. MDS constructs a geometric representation of the data in order to show the degree of the relationship between stimuli that are represented by the data matrix [see Young (Young, 1987) for more details]. In the present study, the 16 visual stimuli were used to elicit neural activity in pulvinar neurons. Data matrices of neural activity in a 91 × 16 array derived from the 91 visually responsive neurons were generated. Euclidean distances as dissimilarity between all possible pairs of 2 visual stimuli were calculated by using the visual responses of the 91pulvinar neurons. Then, the MDS program (PROXSCAL procedure, SPSS statistical package, version 16) positioned the visual stimuli in the 2-dimensional space with the distances between the stimuli representing the original relationships (i.e., Euclidean distances in the present study) (Shepard, 1962; Kruskal, 1964). Then, the clusters of the visual stimuli were evaluated by discriminant analysis.

## Results

### Preferential responses to snakes

Of 745 pulvinar neurons recorded, 105 (14.1%) responded to at least one of the visual stimuli. Of these, 91 neurons were tested with all stimuli. These neurons responded differentially to the categories of visual stimuli. The pulvinar neurons were categorized by the stimulus that elicited the largest responses. “Snake-best” neurons were defined as those in which the mean response to all snake images was the largest among the four stimulus categories. “Face-best”, “hand-best”, and “simple geometrical shape-best” neurons were similarly defined for their respective images. Of the 91 neurons tested, snake-best neurons were most common (n = 37; 40.6%), followed by face-best neurons (n = 26; 28.6%), hand-best neurons (n = 17; 18.7%) and simple geometrical shape-best neurons (n =11; 12.1%) (Fig.3A). The proportion of ‘snake-best neurons’ was significantly larger than those of hand- and simple geometrical figure-best neurons (Chi-square tests, $p<0.01$), and tended to be larger than that of face-best neurons (Chi-square test, $p<0.10$). The proportion of face-best neurons was significantly larger than that of simple geometrical shape-best neurons (Chi-square test, $p<0.05$).

There were also significant differences in mean response magnitudes to the four stimulus categories [repeated measures one-way ANOVA; $F(1, 90)=101.096$, $p<0.001$; Fig. 3B]. Posthoc multiple comparisons indicated that the mean response was significantly greater to snakes than to other stimulus categories (Bonferroni test, $p<0.05$), and that the mean

response magnitude was significantly larger to faces than to simple geometrical shapes (Bonferroni test, $p<0.01$). Differential responses of pulvinar neurons cannot be ascribed to luminance variations in this study since all stimuli employed were controlled for equal luminance and size except the simple geometrical shapes (see Materials and Methods). Furthermore, image scrambling decreased the selective responses to these stimuli (see below), suggesting that these responses were not attributed to local textures, but to the coherent images.

Figure 4A shows an example of a neuron that responded selectively to snakes. This neuron responded strongly to all four snake images (Fig. 4Aa-d) and less to other stimuli (Fig. 4Ae-q). Figure 4B shows response magnitudes of this neuron to all visual stimuli. There was a significant difference among the response magnitudes (one-way ANOVA; $F(15, 177)=13.81$, $p<0.001$). Post-hoc multiple comparisons indicated that the response magnitudes were significantly larger to the snakes than to the other stimuli for this neuron (Tukey test, $p<0.001$). We further analyzed whether shapes of the four snake images (coiled or uncoiled) affected the responses using the 91 pulvinar neurons (Fig. 5). There was no significant difference in mean response magnitudes between the coiled and uncoiled snake images (paired t-test, $p>0.05$).

Most pulvinar neurons responding to the visual stimuli (open circles) were located in the medial (medial pulvinar) and dorsolateral (lateral pulvinar) parts of the pulvinar (Fig. 6). There was no significant difference in the ratio of the neurons responding to the visual stimuli between these two parts of the pulvinar (Chi-square tests, $p>0.05$).

**Response latencies of the pulvinar neurons**

Latencies of pulvinar neuronal responses ranged from 30 to 450 ms. The distribution of the latencies formed two peaks – a short latency group (30 – 120 ms) and a long latency group (170–450 ms). Mean latency of the short latency group was 60.6 ± 2.8 ms, while mean latency of the long latency group was 253.5 ± 26.7 ms. In the short latency group (Fig. 3C), the mean response latency to snakes was very short (55.4 ± 3.4 ms), and was significantly shorter than response latencies to angry faces, neutral faces, hands, and simple geometrical shapes (Bonferroni test after repeated measures one-way ANOVA, $p<0.05$). There was also a significant difference between angry faces and the emotionally nonarousing neutral faces, hands, and simple geometrical shapes (Bonferroni test after repeated measures one-way ANOVA, $p<0.05$).

**Responses to the first stimuli**

In this study, visual stimuli in the same categories were presented in the same blocks. Therefore, habituation to the visual stimuli could potentially occur through repetition of the stimuli of the same categories. To avoid a potential confounding habituation effect, we also analyzed the responses to only the first visual stimulus of each block. Statistical comparison indicated that similar results were obtained (Fig. 7). There were significant differences in mean response magnitudes to the four stimulus categories (repeated measures one-way ANOVA; $F(1, 90)=31.725$, $p<0.001$; Fig. 7A). Post-hoc multiple comparisons indicated that the mean response was significantly greater to snakes than to other stimulus categories (Bonferroni test, $p<0.05$). Furthermore, there were significant differences in mean response latencies to the four stimulus categories (repeated measures one-way ANOVA; $F(1, 78)=178.1$, $p<0.001$; Fig. 7B). Post-hoc multiple comparisons indicated that the mean response to snakes was significantly shorter than to other stimulus categories (Bonferroni test, $p<0.05$).

**Effects of image transformation**

Figure 8A shows an example of a neuron responding to scrambled and filtered images. This neuron responded strongly to the original snake image (Fig. 8Aa). Although low pass filtering did not affect the neuronal firing to the snake image (Fig. 8Ac), scrambling and high pass filtering decreased it (Fig. 8Ab,d). There was a significant difference among the response magnitudes to these stimuli [one-way ANOVA; $F(3, 42)= 11.729$, $p<0.001$; Fig. 8B]. Post-hoc multiple comparisons indicated that scrambling significantly decreased (Tukey test, $p<0.001$) and high pass filtering tended to decrease the response magnitudes to the snake image (Tukey test, $p<0.10$).

A total of 20 neurons were tested with scrambled and filtered snake images in the same way, and Figure 8C displays averaged response magnitudes to these stimuli. Statistical analysis showed a significant difference among the response magnitudes [one-way ANOVA; $F(3, 80)=17.334$, $p<0.001$]. Post-hoc multiple comparisons indicated that both scrambling and high pass filtering significantly decreased the firing rate to the snake image (Tukey test, $p<0.001$ for both comparisons).

**Population coding of snakes by the pulvinar neurons**

The data sets of response magnitudes of the 91 visually responsive pulvinar neurons in epochs 1 (0-50 ms), 2 (50-100 ms) and 3 (100-150 ms) after stimulus onset were subjected to multidimensional scaling (MDS) analysis (Fig. 9). After measurement of $R^2$ and stress value for

up to four dimensions, two-dimensional spaces showed the best results. In the two-dimensional spaces, $R^2$ values of epoch 1, 2 and 3 were 0.843, 0.938 and 0.871, respectively. In epoch 1 (Fig. 9A), two groups were recognized, a cluster containing the snakes and the other containing hands. Discriminant analyses indicated significant separation between snake and hand pictures and between snake and all non-snake stimuli ($p<0.05$) (Table 1). There was also significant separation between hand pictures and simple geometrical shapes ($p<0.05$). In epoch 2 clustering becomes clearer (Fig. 9B). Discriminant analyses indicate significant separations of snakes vs. faces, snakes vs. hands, snakes vs. simple geometrical shapes, and snakes vs. all non-snake stimuli ($p<0.01$). Separations of hands vs. faces, and hands vs. simple geometrical shapes were also significant ($p<0.05$) (Table 1). The results in epoch 3 (Fig. 9C) were similar to those in epoch 2; hands were more clearly separated from the other stimuli ($p<0.01$) (Table 1).

**Discussion**

Primates have substantially modified and expanded the vertebrate visual system, and they rely heavily on vision as their primary sensory interface with the environment. Among other changes, the medial and dorsolateral pulvinar exist only in primates (Preuss, 2007). Major modification of the visual system, including the addition of complex and energetically costly neural components, demands an adaptive explanation.

We show that neurons located especially in the medial and dorsolateral pulvinar respond selectively to snakes and in ways that facilitate their rapid visual detection: 1) the ratio of neurons that responded best to snakes was larger than those of neurons that responded best to other categories, 2) mean response magnitudes were larger to snakes than to other stimuli, and 3) snakes elicited neuronal responses with the shortest latencies. These responses were dependent on low frequency images; high pass filtering of the visual stimuli decreased neuronal responses but low pass filtering did not. Distinct spatial frequencies of visual stimuli convey different information; high spatial frequencies of images convey fine visual details whereas low spatial frequencies encode coarse visual information. Our results provide clear evidence that snakes provide coarse visual information that is effective in eliciting strong and rapid responses from a subset of visually active pulvinar neurons.

The medial and lateral pulvinar have been suggested to assist in shifting attention to relevant visual stimuli (Benevento and Miller, 1981; Benevento and Port, 1995; Stepniewska, 2004). Biologically meaningful stimuli relevant to our primate ancestors must have included snakes (Isbell, 2006; Isbell, 2009; Headland and Greene, 2011). Even today, deadly interactions

with snakes are best avoided with early detection and a shifting of attention to them. The medial pulvinar receives direct inputs from the retina (Preuss, 2007) and the deeper layers of the superior colliculus (Benevento and Fallon, 1975; Stepniewska, 2004). Although the superior colliculus is considered a visual structure, it is also involved in threat-relevant motor behavior. Stimulation of its deeper layers causes animals to turn, dart, or freeze (Sewards and Sewards, 2002), and infant monkeys with bilateral neurotoxic lesions of the superior colliculus continue to reach for food in the presence of a snake model whereas sham-operated monkeys avoid the food (Maior et al., 2011). Evasive movements such as these are typical of animals that are surprised or threatened by others (Ramakrishnan et al., 2005; Carter et al., 2012). The pulvinar is also connected to the amygdala (Jones and Burton, 1976). Studies of humans have implicated a pathway involving the superior colliculus, pulvinar, and amygdala in fast, automatic visual detection of fear-related stimuli, including snakes, at low spatial frequency (Morris et al., 1999; Vuilleumier et al., 2003; Liddel et al., 2005; Csatho et al., 2008; Mulckhuyse and Theeuwes, 2010; Tamietto and de Gelder, 2010; Maior et al., 2012). Similarly, the short response latencies and dependence on low spatial frequency to snakes in neurons of the non-human primate medial and dorsolateral pulvinar that we found corroborate the view that the pulvinar plays a crucial role in quickly conveying essential information affecting survival via bottom-up fast visual information processing (Ward et al., 2005).

This is not to suggest the subcortical route is the only pathway to detecting and avoiding danger. We also found medial and dorsolateral pulvinar neurons with longer latencies, and we suggest that these may receive top-down inputs from cortical visual areas (Olshausen et al., 1993). We also note that both the medial and dorsolateral pulvinar have reciprocal connections with association cortices (Shipp, 2003). Interactive activity via reciprocal connections between subcortical nuclei and cortical areas likely enhances stimulus recognition and attention (Grieve et al., 2002; Pessoa and Adolphs, 2010). Although threatening visual inputs to the pulvinar and conveyed to the amygdala may be quickly processed, the pulvinar also likely coordinates cortical evaluation of the biological significance of affective visual stimuli (Nguyen et al., 2013).

The neuronal response to angry faces, albeit weaker than to snakes, was also faster and stronger than to hands and simple geometrical shapes. As is the case with snakes, being able to quickly detect and evade an angry conspecific undoubtedly has substantial survival value (Headland and Greene, 2011; Öhman et al., 2012). However, because the degree of facial expression in primates varies by body size, phylogenetic history, and group size (as a proxy for

sociality) (Dobson, 2009a, Dobson, 2009b), detecting threat from facial expression alone may not be universal among primates. In contrast, since the origin of primates, snakes have been a universal threat; both primates and snakes that can kill them (i.e., constrictors and venomous snakes) have their greatest diversity in tropical ecosystems (Harcourt, 2006; Ricklefs et al., 2007; Isbell, 2009; Headland and Greene, 2011). Our data provide the first neuronal evidence supporting the hypothesis that snakes provided a novel selective pressure that contributed to the evolution of the primate order by way of visual modification (Isbell, 2006, 2009). We urge neurophysiologists to engage in similar studies across a wide range of primate species and closely related mammals to further examine the phylogenetic fingerprint of fast snake detection.

**References**


1. Asahi T et al (2003) A method for accurate determination of stereotaxic coordinates in single-unit recording studies in monkeys by high-resolution three-dimensional magnetic resonance imaging. *Neurosci Res* 47: 255–260.
2. Benevento LA, Fallon JH (1975) The ascending projections of the superior colliculus in the rhesus monkey (*Macaca mulatta*). *J Comp Neurol* 160:339–361.
3. Benevento LA, Miller J (1981) Visual responses of single neurons in the caudal lateral pulvinar of the macaque monkey. *J Neurosci* 1:1268–1278.
4. Benevento LA, Port JD (1995) Single neurons with both form/color differential responses and saccade-related responses in the nonretinotopic pulvinar of the behaving macaque monkey. *Vis Neurosci* 12:523-544.
5. Carter AJ, Marshall HH, Heinsohn R, Cowlishaw G (2012) How not to measure boldness: novel object and antipredator responses are not the same in wild baboons. *Anim Behav* 84:603-609.
6. Csatho A, Tey F, Davis G. (2008) Threat perception and targeting: the brainstem-amygdalacortex alarm system in action? *Cogn Neuropsychol* 25:1039–1064.
7. Dobson SD (2009a) Allometry of facial mobility in anthropoid primates: implications for the evolution of facial expression. *Amer J Phys Anthropol* 138:70-81.
8. Dobson SD (2009b) Socioecological correlates of facial mobility in nonhuman anthropoids. *Amer J Phys Anthropol* 139:413-420.
9. Grieve KL, Acuña C, Cudeiro J (2000) The primate pulvinar nuclei: vision and action. *Trends Neurosci* 23:35-39.
10. Harcourt AH (2006) Rarity in the tropics: biogeography and macroecology of the primates. *J Biogeogr* 33:2077-2087.
11. Hayakawa S, Kawai N, Masataka N (2011) The influence of color on snake detection in visual search in human children. *Sci Rep* 1: 80.
12. Headland TN, Greene HW (2011) Hunter-gatherers and other primates as prey, predators,and competitors of snakes. *Proc Natl Acad Sci USA* 108:1470-1474.
13. Henshilwood CS, d'Errico F, Yates R, Jacobs Z, Tribolo C, Duller GAT, Mercier N, Sealy JC, Valladas H, Watts I, Wintle AG (2002) Emergence of modern human behavior: Middle Stone Age engravings from South Africa. *Science* 295:1278-1280.
14. Isbell LA (2006) Snakes as agents of evolutionary change in primate brains. *J Hum Evol*

51:1-35.

15. Isbell LA (2009) *The Fruit, the Tree, and the Serpent: Why We See so Well* (Harvard University Press, Cambridge, MA).
16. Itaya SK, Van Hoesen GW (1983) Retinal projections to inferior and medial pulvinar neuclei in the Old-world monkey. *Brain Res* 269:223-230.
17. Jones EG, Burton H (1976) A projection from the medial pulvinar to the amygdala in primates. *Brain Res* 104:142–147.
18. Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29: 1–27.
19. Kusama T, Mabuchi M (1970) *Stereotaxic atlas of the brain of Macaca fuscata* (Tokyo University Press, Tokyo).
20. Liddell BS, Brown KJ, Kemp AH, Barton MJ, Das P, Peduto A, Gordon E, Williams M (2005) A direct brainstem-amygdala-cortical 'alarm' system for subliminal signals of fear. *NeuroImage* 24:235-243.
21. LoBue V, DeLoache JS (2008) Detecting the snake in the grass: attention to fear-relevant stimuli by adults and young children. *Psychol Sci* 19: 284-289.
22. LoBue V, DeLoache JS (2010) Superior detection of threat-relevant stimuli in infancy. *Develop Sci* 13:221-228.
23. Maior RS, Hori E, Barros M, Teixeira DS, Clotilde M, Tavaresa H, Ono T, Nishijo H, Tomaz C (2011) Superior colliculus lesions impair threat responsiveness in infant capuchin monkeys. *Neurosci Lett* 504:257-260.
24. Maior RS, Hori E, Uribe CE, Saletti PG, Ono T, Nishijo H, Tomaz C (2012) A role for the superior colliculus in the modulation of threat responsiveness in primates: toward the ontogenesis of the social brain. *Rev Neurosci* 23:697-706.
25. Masataka N, Hayakawa S, Kawai N (2010) Human young children as well as adults demonstrate 'superior' rapid snake detection when typical striking posture is displayed by the snake. *PLoS ONE* 5:e15122.
26. Matsuda K (1996) Measurement system of the eye positions by using oval fitting of a pupil. *Neurosci Res Suppl* 25: 270.
27. Morris JS, Öhman A, Dolan RJ (1999) A subcortical pathway to the right amygdale mediating "unseen" fear. *Proc Natl Acad Sci USA* 96:1680–1685.
28. Mulckhuyse M, Theeuwes J (2010) Unconscious attentional orienting to exogenous cues: a review of the literature. *Acta Psychol* 134:299-309.

29. Nguyen MN, Hori E, Matsumoto J, Tran AH, Ono T, Nishijo H (2013) Neuronal responses to face-like stimuli in the monkey pulvinar. *Eur J Neurosci* 37:35-51.
30. Nishijo H, Ono T, Nishino N (1988) Single neuron responses in amygdala of alert monkey during complex sensory stimulation with affective significance. *J Neurosci* 8: 3570-3583.
31. Öhman A, Flykt A, Esteves F (2001) Emotion drives attention: detecting the snake in the grass. *J Exp Psychol: General* 130:466-478.
32. Öhman A, Soares JJF (1993) On the automatic nature of phobic fear: conditioned electrodermal responses to masked fear-relevant stimuli. *J Abnorm Psychol* 102:121-132.
33. Öhman A, Soares SC, Juth P, Lindström B, Esteves F (2012). Evolutionary derived modulations of attention to two common fear stimuli: serpents and hostile humans. *J Cogn Psychol* 24:17-32.
34. Olshausen BA, Anderson CH, Van Essen DC (1993) A neurobiological model of visual attention and invariant pattern recognition based on dynamic routing of information. *J Neurosci* 11:4700–4719.
35. Penkunas MJ, Coss RG (2013) A comparison of rural and urban Indian children's visual detection of threatening and nonthreatening animals. *Develop Sci* 16:463-475.
36. Pessoa L, Adolphs R (2010) Emotion processing and the amygdala: from a 'low road' to 'many roads' of evaluating biological significance. *Nat Rev Neurosci* 11:773–782.
37. Preuss TM (2007) *Primate Origins and Adaptations*, eds Ravosa MJ, Dagosto M (Kluwer Academic/Plenum Press, New York), pp. 625-675.
38. Ramakrishnan U, Coss RG, Schank J, Dharawat A, Kim S (2005) Snake species discrimination by wild bonnet macaques (*Macaca radiata*). *Ethology* 111:337–356.
39. Ricklefs R, Losos JB, Townsend TM (2007) Evolutionary diversification of clades of squamate reptiles. *J Evol Biol* 20:1751-1762.
40. Rotshtein P, Vuileumier P, Winston J, Driver J, Dolan R (2007) Distinct and convergent visual processing of high and low spatial frequency information in faces. *Cereb Cortex* 17: 2713-2724.
41. Sewards TV, Sewards MA (2002) Innate visual recognition in vertebrates: some proposed pathways and mechanisms. *Comp Biochem Physiol A Mol Integr Physiol* 132:861-891.
42. Shepard RN (1962) The analysis of proximities: multidimensional scaling with an unknown distance function. *Psychometrika* 27: 125–140.
43. Shibashaki M, Kawai N (2009) Rapid detection of snakes by Japanese monkeys (*Macaca fuscata*): an evolutionarily predisposed visual system. *J Comp Psychol* 123:131-135.

44. Shipp S (2003) The functional logic of cortico-pulvinar connections. *Phil Trans R Soc Lond B* 358:1605–1624.
45. Soares SC (2012) The lurking snake in the grass: interference of snake stimuli in visually taxing conditions. *Evol Psychol* 10:187-197.
46. Stepniewska I (2004) *The Primate Visual System*, eds Kaas, JH, Collins CE (CRC Press, New York), pp. 53-80.
47. Tamietto M, de Gelder B (2010) Neural bases of the non-conscious perception of emotional signals. *Nat Rev Neurosci* 11:697–709.
48. Tazumi T, Hori E, Maior RS, Ono T, Nishijo H (2010) Neural correlates to seen gaze direction and head orientation in the macaque monkey amygdala. *Neurosci* 169: 287-301.
49. Texier P-J, Porrax G, Parkington J, Rigaud J-P, Poggenpoel C, Miller C, Tribolo C, Cartwright C, Coudenneau, A, Klein R, Steele T, Verna C (2010) A Howiesons Poort tradition of engraving ostrich eggshell containers dated to 60,000 years ago at Diepkloof Rock Shelter, South Africa. *Proc Natl Acad Sci USA* 107:6180-6185.
50. Vuileumier P, Armony JL, Driver J, Dolan R (2003) Distinct spatial frequency sensitivities for processing faces and emotional expressions. *Nat Neurosci* 6: 624-631.
51. Vuilleumier P, Armony JL, Driver J, Dolan RJ (2003) Distinct spatial frequency sensitivities for processing faces and emotional expressions. *Nat Neurosci* 6:624-631.
52. Ward R, Danziger S, Bamford S (2005) Response to visual threat following damage to the pulvinar. *Curr Biol* 15:571-573.
53. Young FW (1987) *Multidimensional scaling: History, theory, and applications* (Lawrence Erlbaum, Hillsdale, NJ).

**Figure legends**

**Fig. 1. Visual stimuli (A, B) and delayed non-matching-to-sample (DMNS) task (C) used in the present study.**

(A) Sixteen photos of 4 categories of the stimuli including snakes photos (sn1-sn4) with different head directions (facing to monkeys and attacking toward the sides), faces of two monkeys (f1a, f1b, f2a and f2b) with different emotional expressions (angry and neutral), monkey hands (monkey right and left prone or supine hands: h1-h4), and simple geometrical patterns (s1-s4) (circle, cross, square and star). (B) Scrambling and filtering of visual stimuli. (a) An original snake photo; (b) scrambled image; (c) low-pass filtered (LSF) image; (d) high-pass filtered (HPF) images. (C) Stimulus sequence in the DMNS task in which stimuli were sequentially presented with a delay.

**Fig. 2. Schematic illustration of processes of high pass filter (HPF).**

**Fig. 3. Neurophysiological characteristics of pulvinar neurons.**

(A) Ratio of the neurons that responded best to each stimulus category. **, *, #, significant difference (Chi-square test, $p<0.01$, 0.05, 0.10, respectively). (B) Mean response magnitude to each stimulus category. ***, **, *, significant difference (Bonferroni test after one-way ANOVA, $p<0.001$, 0.01, 0.05, respectively). (C) Mean response latency to each stimulus category. ***, **, *, significant difference (Bonferroni test after one-way ANOVA, $p<0.001$, 0.01, 0.05, respectively).

**Fig. 4. An example of a pulvinar neuron that responded most strongly to snakes.**

(Aa-q) Raster displays of neuronal activities and their summed histograms in response to each stimulus. (a-d) responses to snakes, (e-h) responses to monkey faces, (i-l) responses to monkey hands, and (m-q) responses to simple geometrical shapes. Horizontal bars above the raster displays indicate the stimulus presentation periods (500ms). Vertical line in each of the raster displays and histograms indicates the stimulus onset. Calibration at the right bottom of the figure indicates the number of spikes per trial in each bin. Bin with = 50ms. (B) Response magnitudes of the neuron shown in (A) to the 16 visual stimuli. The neuron responded most strongly to the snakes (Tukey test after one-way ANOVA, $p<0.001$).

**Fig. 5. Mean response magnitudes to the coiled and uncoiled snake photos.**

Statistical comparison indicated that there was no significant difference in response magnitudes (paired t-test, $p>0.05$).

**Fig. 6. Stereotaxic plots of the pulvinar neurons on the MRI photo of the monkey brain.**

The 745 pulvinar neurons were recorded from AP 8.0 to AP 5.0, but plotted on the plane at AP 7.0. The number in the left upper corner indicates the distance (mm) anteriorly from the interaural line. The horizontal axis indicates the distance (mm) from the midline; vertical axis indicates the distance (mm) from the interaural line. Open circles, visually responsive neurons; dots, nonresponsive neurons.

**Fig. 7. Mean response magnitude (A) and latency (B) to each stimulus in each category presented in the first trial of the block.**

*, significant difference (Bonferroni test after repeated one-way ANOVA, $p < 0.05$).

**Fig. 8. Effects of scrambling and filtering of the images.**

(A) An example of neuronal responses to the original (a), scrambled (b) and filtered [c (low pass filtering), d (high pass filtering)] images (same neuron shown in Fig. 1). (B) Response magnitudes to the stimuli shown in A. Scrambling significantly decreased (Tukey test after one way ANOVA, $p<0.001$) and high-pass-filtering tended to decrease the responses to the original image (Tukey test after one-way ANOVA, $p<0. 10$). (C) Mean response magnitudes to the scrambled and filtered images (n=20). Scrambling and high-pass-filtering significantly decreased the responses to the original image (Tukey test after one-way ANOVA, $p<0.001$).

**Fig. 9. Mulidimentional scaling analyses of the pulvinar neuronal responses.**

Distributions of the 16 visual stimuli in a two-dimensional space resulting from multidimensional scaling using responses of the 91 neurons to these stimuli in epoch 1 (A), epoch 2 (B), and epoch 3 (C). In epochs 1 and 2 (A, B), the snakes were separated from the remaining stimuli. In epoch 3 (C), 3 groups were separated: snakes, hands, and a cluster containing the faces and simple geometrical shapes.

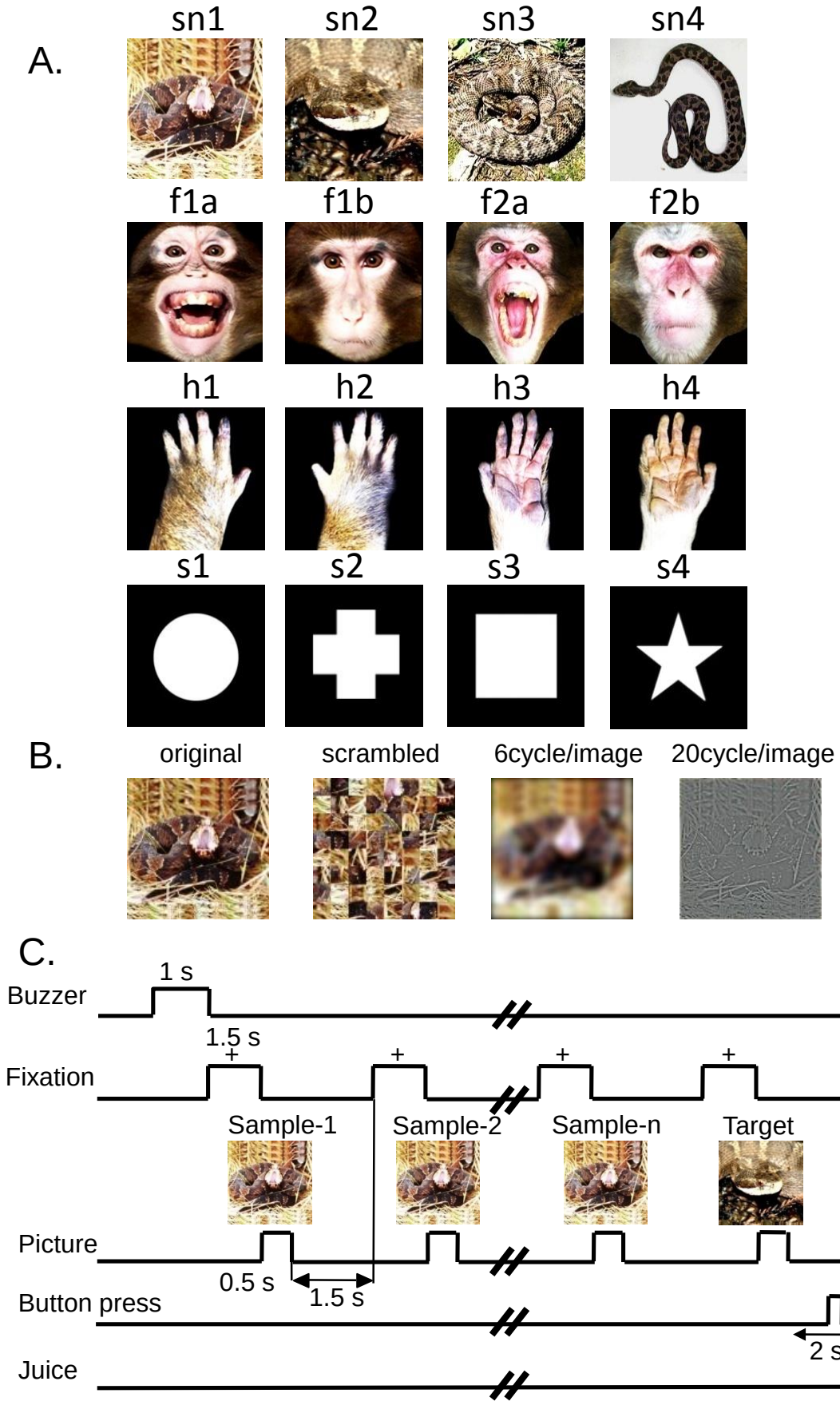


Figure 1. Visual stimuli

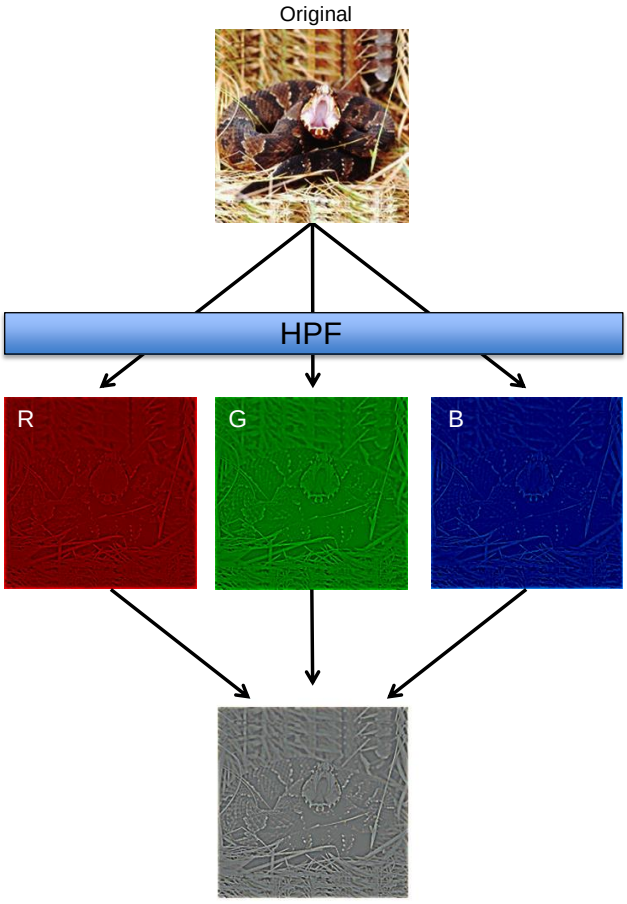


Figure. 2 Processing of filtering images

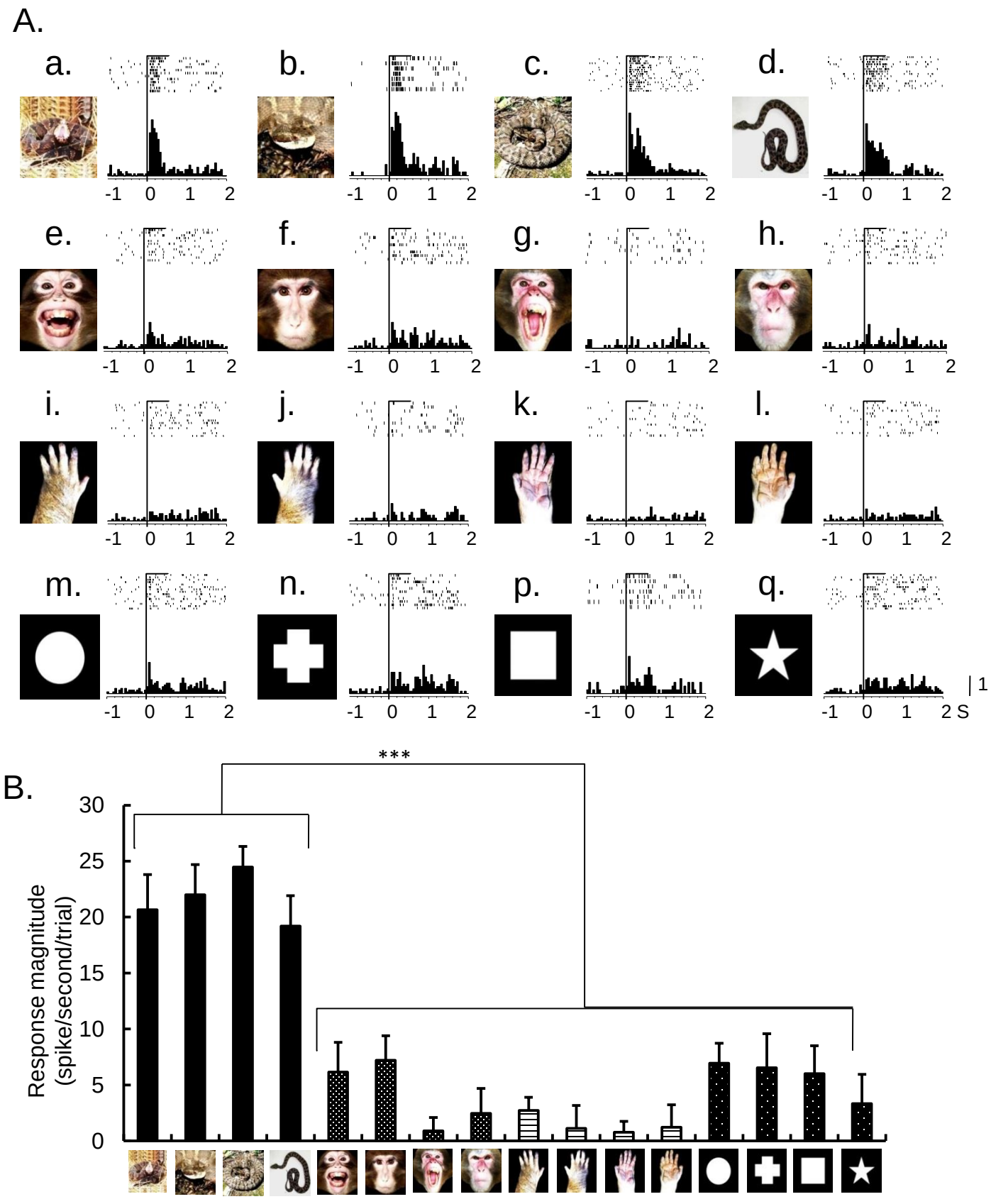


Figure 3. An example of a neuron respond selectively to snakes

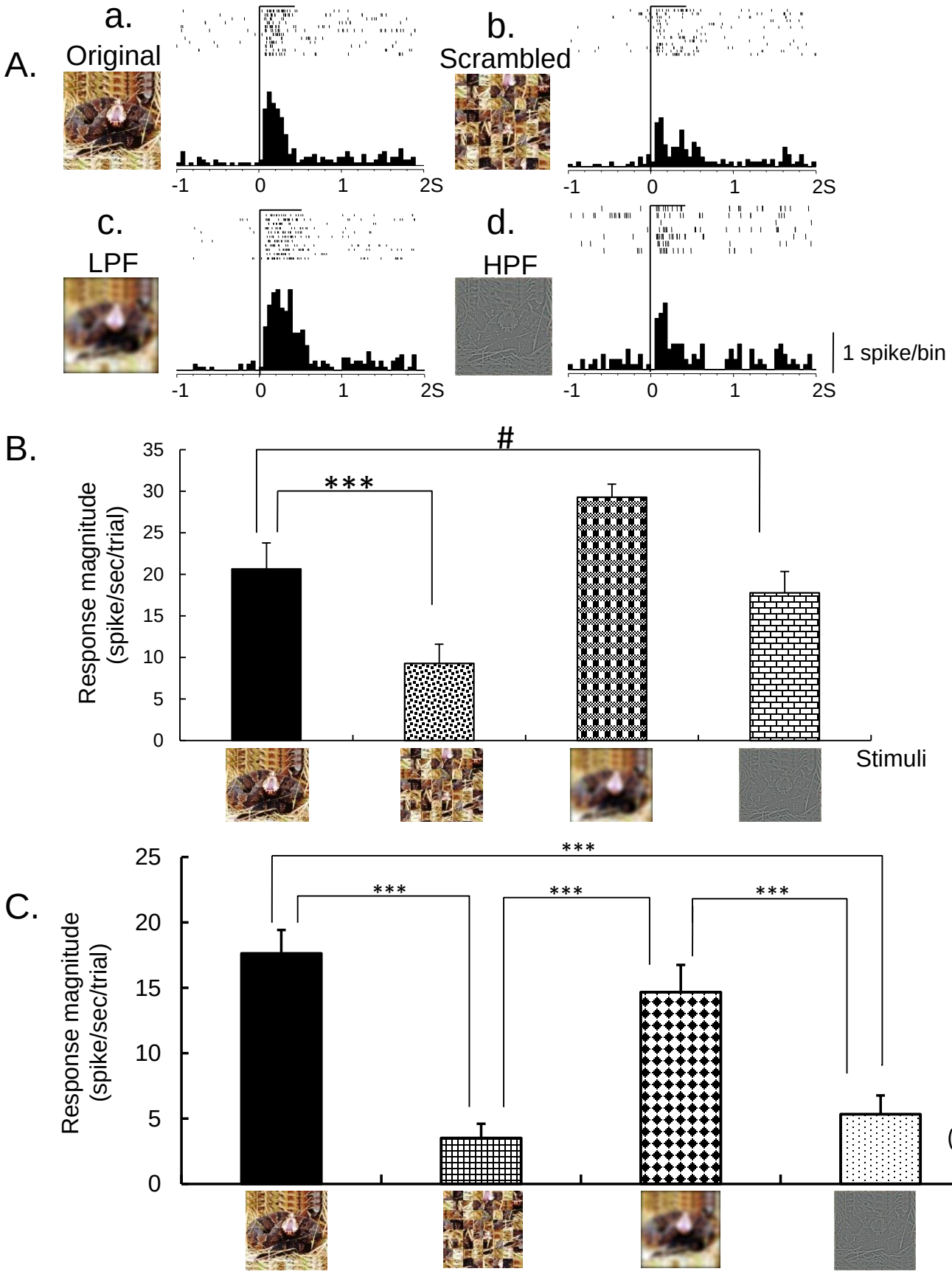


Figure 4. A neuron responds to scrambled and filtered images

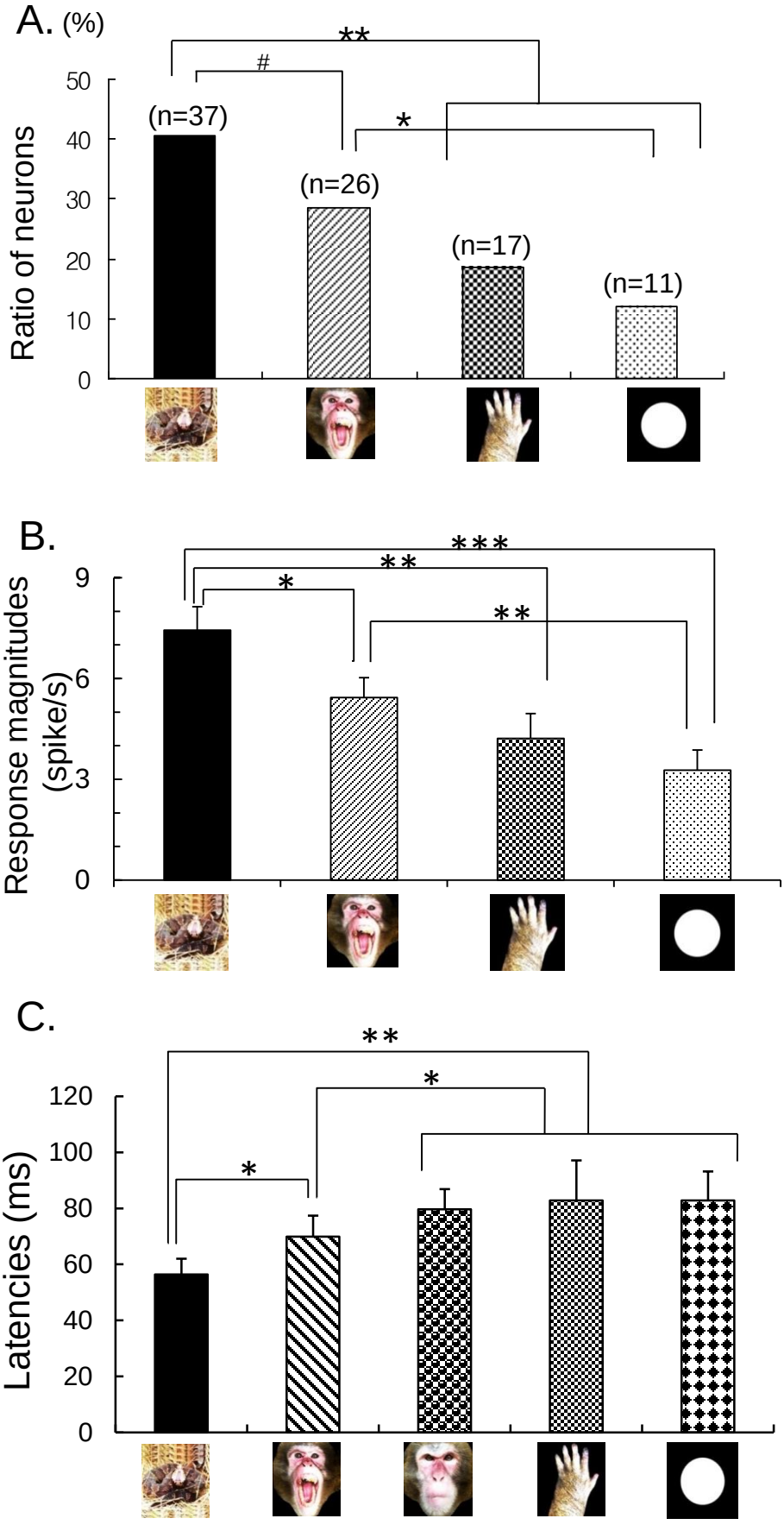


Figure 5. Neurophysiological characteristics of pulvinar neurons

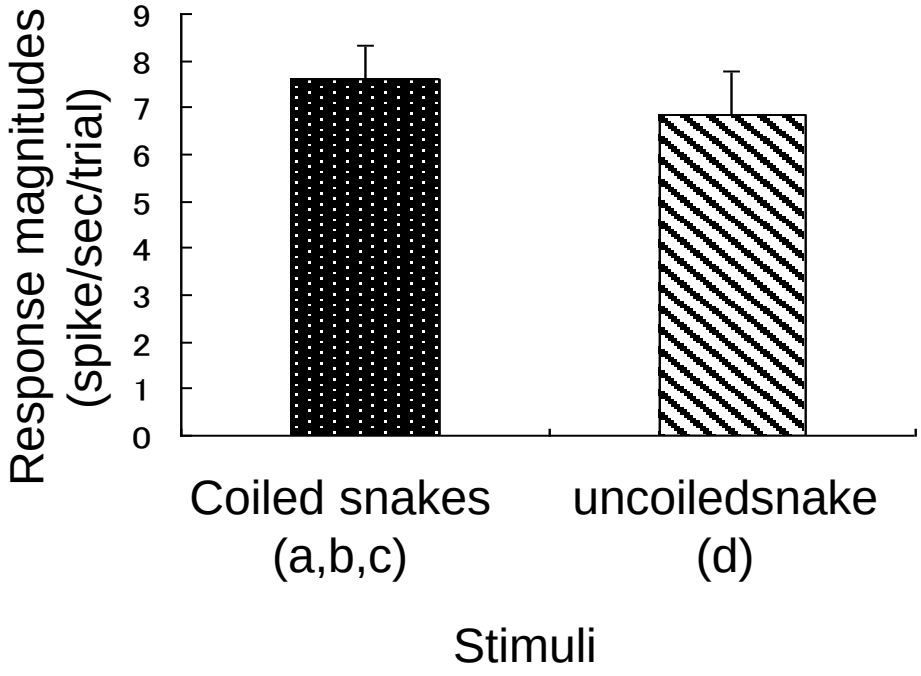


Figure 6 mean responses to coiled snakes and uncoiled snakes

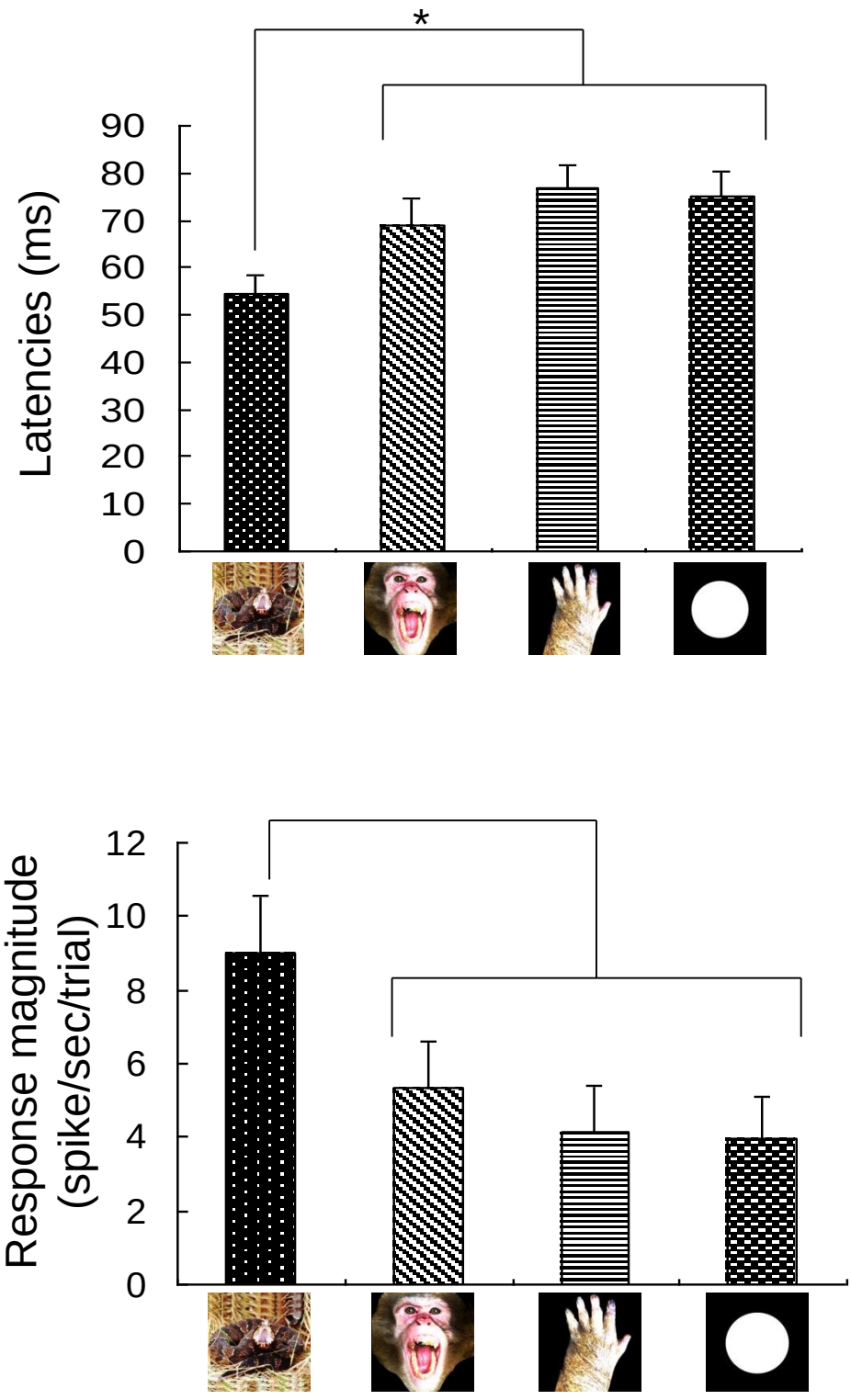


Figure 7. Mean responses and latencies to visual stimuli in the first trials

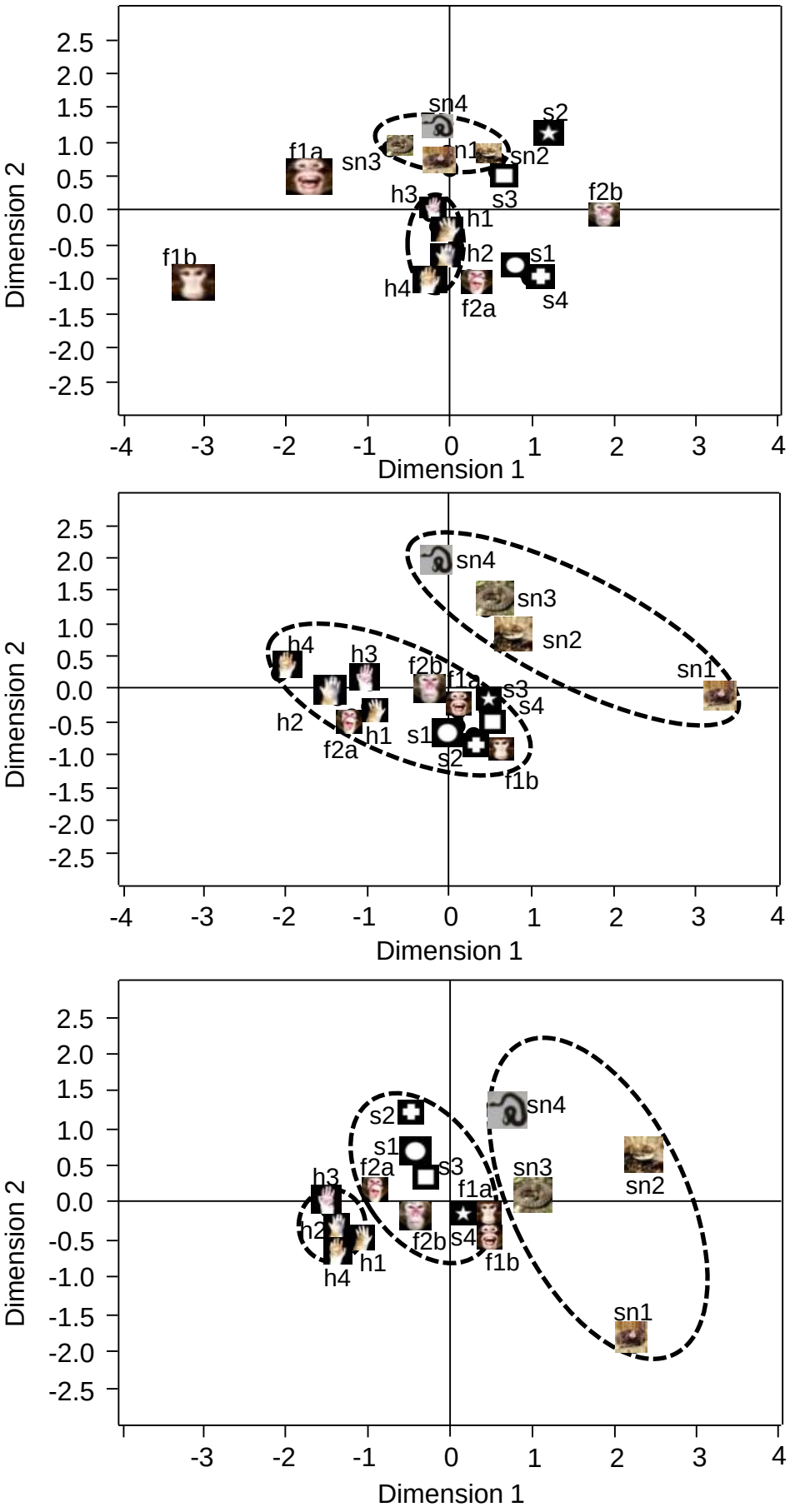


Figure 8. Multidimensional scaling analysis

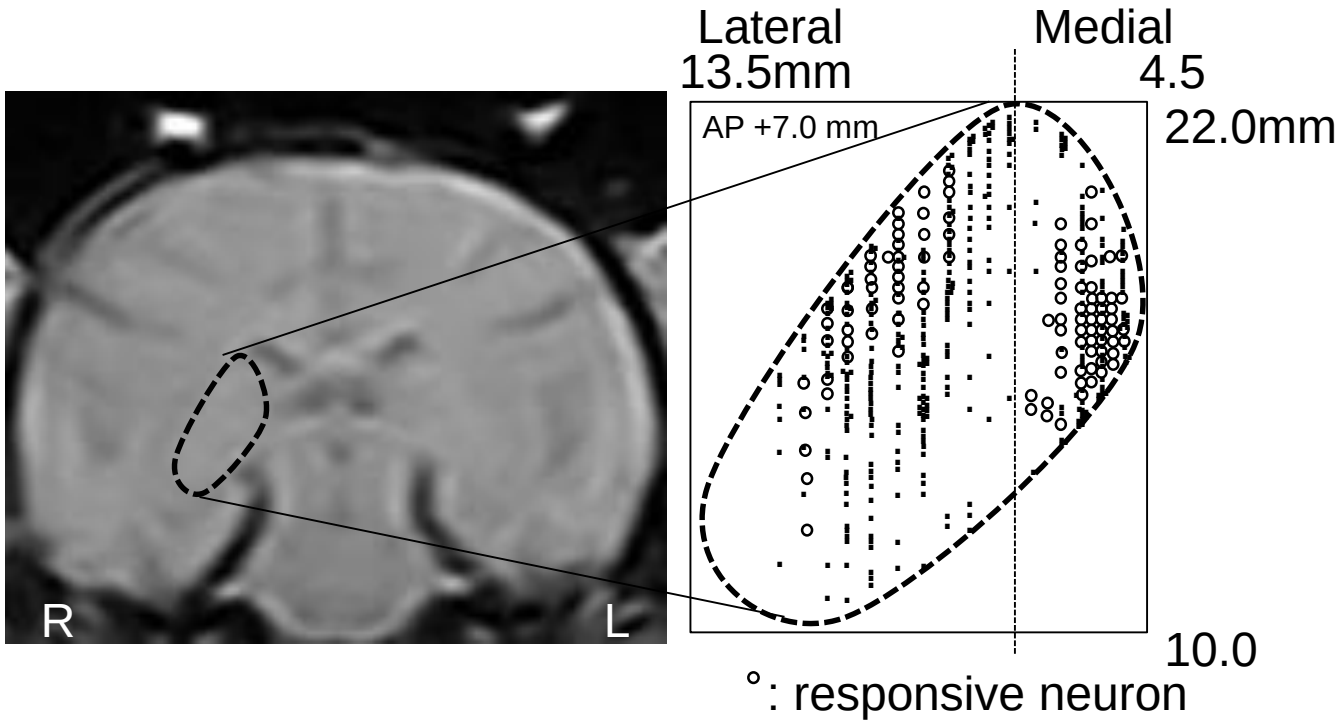


Figure 9. Sterotaxic plots of the pulvinar neurons on the MRI photo of the monkey brain

# Table 1. The discriminant of groups

| Group | $R^2$ | Correct ratio | p |
|---|---|---|---|
| Epoch 1 | 0.843 (MDS) | 50 | **0.12** |
| Snake-face | | 87.5 | 0.096 |
| snake-hand | | 100 | **0.014** |
| snake-simple | | 87.5 | 0.06 |
| snake-non snake | | 81.3 | **0.037** |
| face-hand | | 62.5 | 0.852 |
| face-simple | | 87.5 | **0.451** |
| hand-simple | | 100 | **0.003** |
| Epoch2 | 0.938 (MDS) | 68.8 | **<0.001** |
| Snake-face | | 100 | **0.003** |
| snake-hand | | 100 | **<0.001** |
| snake-simple | | 100 | **0.002** |
| snake-non snake | | 100 | **<0.001** |
| face-hand | | 87.5 | **0.015** |
| face-simple | | 62.5 | **0.486** |
| hand-simple | | 100 | **0.007** |
| Epoch3 | 0.871 (MDS) | 93.8 | **<0.001** |
| Snake-face | | 100 | **0.06** |
| snake-hand | | 100 | **0.003** |
| snake-simple | | 100 | **0.04** |
| snake-non snake | | 100 | **0.001** |
| face-hand | | 100 | **0.005** |
| face-simple | | 87.5 | **0.19** |
| hand-simple | | 100 | **<0.001** |
| snake_facesimple | | 100 | **0.004** |
| hand_facesimple | | 100 | **<0.001** |