Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

	2019. 04, 29
Affiliation/Position	Primate Research Institute/D1
Name	Vanessa Nadine Gris

1. Country/location of visit

Koshima, Miyazaki Prefecture, Japan

2. Research project

Koshima Field Course

3. Date (departing from/returning to Japan)

2019. 04. 21 – 2019. 04. 27

4. Main host researcher and affiliation

Professors Yamato Tsuji and Hiroyuki Tanaka

5. Progress and results of your research/activity (You can attach extra pages if needed)

Please insert one or more pictures (to be publicly released). Below each picture, please provide a brief description.

From April 21 to 29 I joined Koshima field course. We had the chance to observe Japanese macaques in the wild and semi-wild horses in Cape-Toi. We also developed individual projects and mine is described below.

Do Japanese macaques respond to facial expressions of pain?

Introduction

According to the International Association for the Study of Pain (IASP), pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue injury. The IASP reinforces that pain is subjective and that the inability to communicate verbally does not eliminate the possibility of an individual is experiencing pain (Paul-Murphy et al., 2004). Since all vertebrates have the components for nociception of pain and the role of components for the perception of noxious stimuli is still not well established, animals should be considered to experience aversive pain or feel pain *per se*, even though they may not verbally communicate their consciousness (Hawkins, 2006).

Facial expressions work as nonverbal signals of pain are useful in a social context. In non-human primates, the facial expressions combined with auditory cues or other physical signals can alert the individuals of the group about potential environmental harm, such as the presence of predator or non-familiar individuals (Hammerschmidt & Todt 1995; Sebe et al. 2010). Humans show a bias to attend preferentially to signals of threat, such as angry faces compared with neutral faces (Holmes et al, 2009). As stated, this can be beneficial in terms of faster detection of threat and therefore improved ability to defend against, or escape, danger (Ohman & Mineka, 2001).

In this study Japanese macaques were exposed to different pictures of conspecifics (one with a potential painful and one with a neutral expression). We predicted that the Japanese macaques will respond more strongly to the pictures showing painful faces.

Material and methods

Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

The study was conducted in April 2019 on Japanese macaques living in Koshima Island, Miyazaki Prefecture, Japan (31°22'N and 131°23'E). At the time we conducted the study, the population of macaques consisted of 2 troops of approximately 80 individuals each. Observations were recorded over two days from 9:30 to 13:00 in the sand area. We recorded videos using three cameras: one closer to the face of the animals (GoPro) and the other set about 1.5 to 2 meters of distance for posterior identification of the pictures (Panasonic). The third camera (GoPro) was used to record spontaneous reactions of the animals from other angles and was carried by experimenter 2.

Twenty portraits of adult Japanese macaques (males and females) were shown to the animals. Ten of them portrayed a neutral facial expression (NP) and the other ten presented facial expression after a potential painful situation (P) (Figure 1). All of the pictures were taken from Japanese macaques belonging to Kyoto University Primate Research Institute. Pictures were selected from videos recorded in the institution before (NP) and after surgical procedures or natural injuries (P). Faces were then cropped and printed in A4 thick white paper.

P

NP

Fig. 1 Example of portrait photographs of the two tested groups used as stimuli (P Pain, NP NoPain)

Experimental design

The experimenter 1 sat on a rock and hold one picture in each hand, about 1 meter from each other. One of the pictures displayed a pain face and the other a neutral face. Pictures were always shown in sets of two, totalizing 10 trials. Experimenter 2 placed small amounts of wheat in front of the cameras as an incentive for the animals to approach. The observers tried to drew the attention of the subjects by tapping on the papers and whistling. Observers wore hats to avoid direct eye contact with the macaques. Experimenter 1 one did not participate in the previous selection of pictures and was unaware whether the picture was showing a pain or neutral face.

Recorded began when the animal approached the pictures and ended when the subject left the recording area or was outside the delimited area of study. A trial was aborted if the subject moved away permanently within the first 10 s after trial start. Animals were identified later by screenshots of the videos. The monkeys attended the trials voluntarily and were not trained to perform any activity.

Video analysis

Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

Video from experimental sessions was coded by one observer using the programs BORIS (Friard & Gamba, 2016). Videos were converted to mp4 files and analyzed in slow motion. Video was coded for: arrival and leave of the animal, change of picture set, direction of gaze (either towards the painful face or the pain free face)

Statistical analysis

The statistical analyses were performed using the software RStudio (2009-2016 RStudio, Inc.). We ran a generalized linear mixed model to study the effect of the type of picture shown to the individuals on the proportion of time spent looking at them. These proportions were accounting for how long each pair of pictures was displayed/visible. After removing the outliers from our dataset, we set the type of picture (Pain versus No Pain) as a fixed effect and the identity of the individual as a random effect (random intercept model). The proportion of time watching a picture was set as a dependent variable, following a binomial distribution (logistic regression), in regard of the time the pair of pictures was shown, which was set as a weight. We then inspected residual plots to confirm the absence of pattern or heteroskedascity.

Results

We analyzed approximately 10 hours of mp4 video files and collected 85 observations. At first, six subjects were chosen based on age and gender (number of observations = 175). After initial statistical tests, we decided to remove 2 individuals from the data set. In total, we tested 4 subjects: three adults (Kibana, Shide and Tsuwa) and one juvenile female (under 5 years old; Tabu) and found that the proportion of time spend looking at pictures displaying pain was significantly higher than the one looking at non pain ones (GLMM, $p < 0.05$) (Table 1; Figure 2).

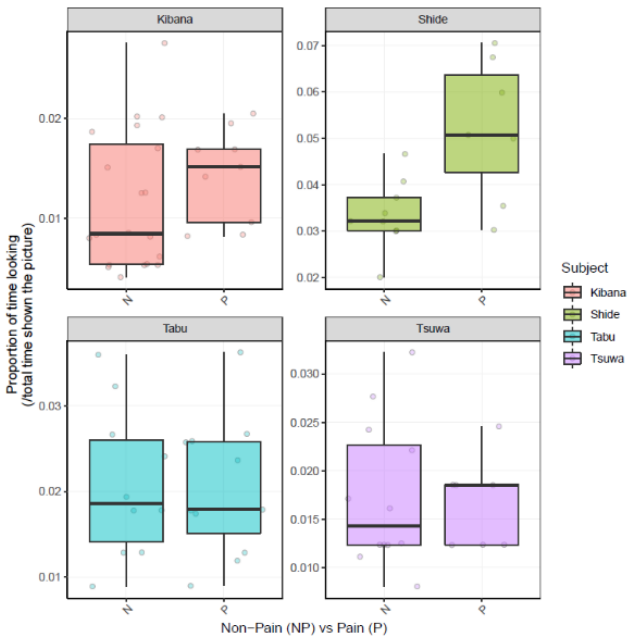

Fig. 2 Japanese macaques (n = 4) proportion of time looking to
pictures showing painful (P) or neutral facial expressions (NP) in Koshima Island

	Estimate	Std. Error	Error z	Pr(>|z|)
No Pain	-3.9896	0.2412	-16.54	<2e-16 ***
Pain	-3.7677	0.2412	-15.62	<2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Fig. 2 Japanese macaques (n = 4) spontaneous time observation of pictures showing painful or neutral facial expression in Koshima Island

Discussion

The troop included in this study was habituated to humans, allowing close-up video recording, but macaques did not try to touch the pictures or get to close to the observers (other than for food provision). On the first trial day, we distributed some peanuts. The macaques are regularly fed with wheat, so peanuts seemed to be a more interesting option to attract them to the perimeter of the study. The trick greatly attracted the attention of the alpha male, who would not leave the area of experiment and also block the approach of other subjects. From the second day we provided them wheat and very rarely, peanuts, and we managed to keep the alpha male away for longer time.

The experiments revealed that Japanese macaques show a greater interest in pictures of animals with facial expressions of pain compared to pictures of neutral faces. One possible explanation why the macaques would respond more strongly to the pain expression pictures is because they recognize it as advice of potential harm.

Depiction of pictures of kin and non-kin to Barbary macaques (*Macaca sylvanus*) demonstrates that adults show a greater interest in pictures of animals from a neighboring group compared to pictures of conspecifics from their own group. One possibility is that they recognize the outsiders as potentially hostile (Schell et al., 2011). Pfefferle et al, (2014) obtained similar results in their study with free-ranging rhesus macaques (*Macaca mulatta*) in the Cayo Santiago island. Also, captive rhesus macaques showed initial vigilance and sustained attention towards aggressive vs neutral expression when presented to face images (Bethell et all., 2012).

We also noticed that the animals stared at the pictures for just a few seconds before looking away. Several primate species perceive staring as a sign of aggression and avoid it to minimize conflict. For bonnet macaques (*Macaca radiata*), the latency and duration of gaze avert differs according to the age and the environment. In the forest juveniles tend to establish eye contact with older males for longer periods of time than did adults, but not in urban sites (Coss et al., 2002). This was not evaluated in our study, although we notice that younger individuals seemed more active and agitated compared to the adults.

It is importance to notice that we had results from a small number of individuals and limited time of recording. Although our hypothesis was confirmed, the p-values were very small. This could possibly be a case of a model that is overfitted. It can still indicate that this trend could be a real phenomenon, but more data is necessary. Also, video analysis was performed by a single observer. Once the precision of the video coding procedure is very significant in the final values, we should have one more coder to verify reliability.

The data we analyzed so far and the methods we tested served as a good pilot study for further investigations. We would like to increase the number of subjects and investigate other variables such as latency for the first gaze, preferential direction of the first gaze and the total duration. With a greater number and variability of individuals it would be interesting to test the for differences in sex, age and social hierarchy.

We concluded that Japanese macaques tend to spend more time looking at pictures where individuals express pain. However, the study requires a larger number of individuals and further comparisons taking into account the different subjects. The quality of the photographs (i.e. luminosity) and inter-observer reliability for the video coding should also be considered.

References

Bethell EJ, Holmes A, MacLarnon A, Semple S (2012) Evidence That Emotion Mediates Social Attention in Rhesus Macaques. PLoS ONE 7(8): e44387.

Coss RG, MARKS S, RAMAKRISHNAN U. Early Environment Shapes the Development of Gaze Aversion by Wild Bonnet Macaques (Macaca radiata) (2002). Primates, 43(3): 217-222

Friard O. and Gamba M. (2016), BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol Evol, 7: 1325–1330.

Hammerschmidt K, Todt D (1995) Individual differences in vocalizations of young Barbary macaques (Macaca sylvanus): a multiparametric analysis to identify critical cues in acoustic signal-
ling. Behaviour 132:381–399

Hawkins, M. G. The Use of analgesics in birds, reptiles, and small exotic mammals. Journal of Exotic Pet Medicine. [S.l.], v. 15, n. 3, p. 177-192, 2006.

Holmes A, Bradley BP, Kragh Nielsen M, Mogg K (2009) Attentional selectivity for emotional faces: Evidence from human electrophysiology.
Psychophysiology 46: 62–68

Ohman A, Mineka S (2001) Fears, phobias, and preparedness: Toward an evolved module of fear and fear learning. Psychological Review 108: 483–522.

Paul-Murphy J, Robertson SA, Gaynor JA, Hellyer PW, Wong PL (2004). The need for a cross-species approach to the study of pain in animals. Journal of the American Veterinary Medical Association, Schaumburg, v. 224, n. 5, p. 692-697.

Pfefferle D, Kazem AJN, Brockhausen RR, Ruiz-Lambides AV, Widdig A (2014). Monkeys Spontaneously Discriminate Their Unfamiliar Paternal Kin under
Natural Conditions Using Facial Cues. Current Biology 24, 1806–1810

Sebe F, Duboscq J, Aubin T, Ligout S, Poindron P (2010) Early vocal recognition of mother by lambs: contribution of low- and high-frequency vocalizations. Anim Behav 79:1055–1066.

Schell A, Rieck K, Schell K, Hammerschmidt K, Fischer J. Adult but not juvenile Barbary macaques spontaneously recognize group members from pictures
Anim Cogn (2011) 14:503–509

Williams A. Facial expression of pain: An evolutionary account (2002). The Behavioral and brain sciences. 25. 439-55; discussion 455.

6. Others

I would like to thank my supervisor for the material and support in studying this subject; Prof. Prof. Hiroyuki Tanaka and Prof. Yamato Tsuji for conducting the course; Cátia Correia Caeiro for collaboration in the experiment; Takafumi Suzumura for identifying the individuals, help in the station and provide the wheat grains; Kenneth Keuk for teaching me the statistical analysis and students that made this field course a very pleasant experience. I am grateful for the financial assistance provided by the Leading Graduate Program in Primatology and Wildlife Science (PWS), Kyoto University.