Understanding of Others in Chimpanzees (*Pan troglodytes*):

Cognitive and Affective Underpinnings

Yutaro Sato

2021

Abstract

The cognitive abilities enabling the understanding of others are important for guiding individuals' behavior adaptively in complex societies. Previous studies have reported pronounced abilities to understand others in chimpanzees (*Pan troglodytes*). I conducted experiments with chimpanzees to investigate the cognitive and affective processes related to the understanding of others, particularly when others exhibit unusual bodily cues and react to disturbing situations. Specifically, I assessed chimpanzees' visual attention to stimuli in eye tracking experiments. In addition, I aimed to probe the role of affective processes using physiological measurements and test an alternative hypothesis involving a simple affective process. I examined 1) chimpanzees' gaze and physiological responses toward others' injury; 2) whether chimpanzees distinguish impossible body movements from possible movements to assess their knowledge about biomechanics; and 3) chimpanzees' association between other conspecifics' vocalizations (i.e., alarm and food-associated calls) and relevant objects in the environment (i.e., snakes and fruits). I discussed the findings from the three approaches in relation to possible differences between chimpanzee and human cognition. The current findings will be useful for informing future investigations of understanding of others in chimpanzees and other nonhuman animals.

Keywords: Chimpanzee; Understanding of others; Eye tracking; Thermal imaging

Contents

Note on Archiving

Chapter 2 is based on the following article. Reproduced with permission from Springer Nature.

Sato Y, Hirata S, Kano F (2019) Spontaneous attention and psycho-physiological responses to others' injury in chimpanzees. Anim Cogn 22:807–823. https://doi.org/10.1007/s10071-019-01276-z

Chapter 3 is based on the following article. Reproduced with permission from Springer Nature.

Sato Y, Kitazaki M, Itakura S, Morita T, Sakuraba Y, Tomonaga M, Hirata S (2021) Great apes' understanding of biomechanics: eye-tracking experiments using three-dimensional computer-generated animations. Primates 62:735–747. https://doi.org/10.1007/s10329-021-00932-8

Chapter 4 is based on the following article. ©American Psychological Association, [2021]. This paper is not the copy of record and may not exactly replicate the authoritative document published in the APA journal. The final article is available, upon publication, at: https://doi.org/10.1037/com0000305

Sato Y, Kano F, Morimura N, Tomonaga M, Hirata S (2021) Chimpanzees (*Pan troglodytes*) exhibit gaze bias for snakes upon hearing alarm calls. J Comp Psychol. Advance online publication.

Chapter 1

General Introduction

1.1 Understanding of Others in Chimpanzees

Some species of primates live in complex societies involving high levels of socio-cognitive demands, such as the need for behavioral coordination and the maintenance of multiple relationships among individuals (e.g., Dunbar and Shultz 2017). This characteristic is proposed to have had a substantial impact on the evolution of large brains in primates (for a recent review, see Dunbar and Shultz 2017, although various perspectives have been proposed: e.g., Barrett et al. 2007; Barrett et al. 2022). Species living in more complex societies need to cope with great uncertainty about others' upcoming social behaviors (Whiten 2000) as well as their affective and cognitive states, hence involving greater social entropy (Freeberg et al. 2019). Thus, the cognitive abilities required to understand others may play an important role in guiding adaptive behaviors according to each social condition, which may bring adaptive benefits by, for example, fostering affiliation and social bonds with groupmates (Seyfarth and Cheney 2013).

Cognitive processes associated with understanding of others in nonhuman animals, including chimpanzees (*Pan troglodytes*), have long garnered interest from comparative psychology researchers. Some facets of these cognitive processes have been explored in depth, including understanding of others' affective states (reviewed in Ferretti and Papaleo 2018; Kim and Kret 2022; Nieuwburg et al. 2021) and the mental states underlying observed actions (e.g., intention and perspectives) (reviewed in Lewis and Krupenye in press; Krupenye and Call 2019; Bettle and Rosati 2021), as described briefly in the following paragraphs. Moreover, animal empathy, as a research topic that encompasses these cognitive processes (reviewed in Adriaense et al. 2020; Brooker et al. in press; de Waal and Preston 2017), has received a recent surge of interest (Watanabe 2017).

Previous studies have revealed that some animal species can react appropriately in accordance with others' affective states expressed through species-typical cues (e.g., facial and vocal expressions) (reviewed by Ferretti and Papaleo 2018; Kim and Kret 2022; Nieuwburg et al. 2021). For example, a previous experiment suggested that captive chimpanzees were able to match conspecific facial expression with corresponding contexts (Parr 2001). In that study, chimpanzees were presented with video clips depicting affective scenes and subsequently with two photos of conspecific facial expressions. Three chimpanzee participants readily learned to select facial expressions that matched the preceding videos in terms of affective valence (e.g., videos of a conspecific being injected were matched with negative facial expressions). Another study suggested that captive apes, including chimpanzees, were able to discriminate emotional expressions of humans and guide their decision-making appropriately (Buttelmann et al. 2009). This finding highlights chimpanzees' ability to understand the association between affective states and facial expressions, even in heterospecific animals such as humans.

Furthermore, numerous studies have examined how animals understand the mental states underlying others' actions (e.g., intention and perspectives) (reviewed by Lewis and Krupenye in press; Krupenye and Call 2019; Bettle and Rosati 2021). Recent studies suggest that apes may even attribute beliefs to others when observing others' actions (Buttelmann et al. 2017; Kano et al. 2019). One study reported that apes were able to attribute false-belief to others while ruling out an alternative interpretation of data based on a simpler mechanism (Kano et al. 2019). In that study, captive apes, including chimpanzees, were presented with movies depicting a human experimenter's actions while their gaze was measured with an eye tracker. Apes were found to show a pattern of gaze that indicated their expectation of the experimenter's action. Before apes saw the test movies, half of the apes were presented with an opaque barrier, while the others were presented with a similar looking but see-through barrier. A similar barrier was also depicted in the movies. Apes were found to take the properties of the barrier they had experienced themselves into account when they directed their gaze to the target of the human experimenter's action, which suggested that apes were not merely tracking the behaviors of the experimenter. In addition, Buttelmann et al. (2017) also found evidence for false-belief understanding in apes, and they suggested that apes are able to use this understanding to guide their behavior to help a human experimenter.

Animal empathy is an overarching research area encompassing these topics and has received much research attention (reviewed by Adriaense et al. 2020; Brooker et al. in press; de Waal and Preston 2017). To date, chimpanzees have been suggested to possess the most complex forms of empathy among nonhuman animals, possibly involving multifaceted cognitive and affective mechanisms, although this view may have partially resulted from the substantial research effort devoted to this species (Yamamoto 2017). For example, chimpanzees were found to help a conspecific partner by handing over an appropriate tool to acquire food rewards (Yamamoto et al. 2012). In that study, five chimpanzees were paired with a partner individual (i.e., their mother or offspring), and a pair of chimpanzees was kept in two separate rooms. An apparatus was attached to the room of the partner individual, from which they were able to obtain a food reward (i.e., juice) if they used an appropriate tool (either a stick or a straw depending on the condition), whereas the participant individual was given seven objects, including the one that their partner needed. The chimpanzees were found to hand over the appropriate tool to the partner, albeit usually in response to the partner's request behaviors (e.g., reaching arm) rather than spontaneously helping. This finding suggested that chimpanzees can assist others to achieve a goal on the basis of understanding others' situation. The findings also underscored the importance of direct requests for chimpanzees to help others (Yamamoto et al. 2012). Interestingly, another study also suggested that chimpanzees rarely helped via such tool transfers irrespective of partners' requests when they could also perform the task themselves (Nolte and Call 2021).

The ability to understand others has been observed in contexts in which processing of visual information plays a dominant role, and also in contexts where auditory information, such as conspecific vocal signals, is crucial (reviewed in Nieuwburg et al. 2021). For example, one field study compared wild chimpanzees' behavioral reactions to screams emitted in severe or mild aggressive interactions using a playback experiment (Slocombe et al. 2009). Chimpanzees looked in the direction of a hidden speaker for longer when they heard a scream that was emitted in a severe aggressive interaction compared with when they heard a scream that was emitted in a mild aggressive interaction, suggesting that chimpanzees may associate others' screams with the severity of corresponding aggressive events. Moreover, chimpanzees looked in the direction of the speaker faster, more often, and for longer when they heard screams that were emitted in a severe aggressive interaction compared with when they heard an acoustically similar but less socially relevant call (i.e., an infant's tantrum scream), suggesting that chimpanzees were not simply reacting to particular acoustic features. Furthermore, a field playback experiment revealed that wild chimpanzees were more likely to emit alarm calls in response to a snake model after hearing playback of rest calls (indicative of the simulated conspecific's ignorance of the imminent threat) compared with after hearing alarm calls (indicative of the simulated conspecific's awareness of the threat), which suggested that chimpanzees emit alarm calls depending on the possible receivers' knowledge about the snake (Crockford et al. 2017). This large body of evidence illustrates chimpanzees' pronounced capacity to understand others, in both the visual and auditory modalities.

Moreover, various behavioral reactions have often been documented when chimpanzees observe others in unusual, irregular conditions (e.g., O'Connell 1995). For example, chimpanzees have occasionally been observed to direct affiliative behaviors toward injured conspecifics (e.g., Clark et al. 2021). A chimpanzee was observed to carefully remove a snare from a caught individual (Amati et al. 2008). A preliminary finding indicated that chimpanzees were able to distinguish humans who were feigning injury, finding that they gazed at the human experimenter and reduced water-spitting behaviors toward them (Itakura 1994). In contrast, they were also observed to exhibit fearful reactions toward a festering wound of an individual (Goodall 1986a, Chapter 13). In a similar vein, two observations were reported in cases of individual chimpanzees with polio, whose peculiar movements elicited fear, avoidance, puzzlement, and aggression from groupmates upon first encounter, with some individuals subsequently approaching them over the following days (Goodall 1986b). Chimpanzees were also observed to exhibit fearful and excited reactions toward anesthetized conspecifics being carried by humans (Hebb 1946). A recent report described in detail an encounter between several chimpanzees and a mother chimpanzee carrying an infant with albinism (Leroux et al. 2021). Some chimpanzees were observed to exhibit possible signs of fear and confusion, including alarm calls, and after infanticide of the infant, several individuals approached the carcass (Leroux et al. 2021).

Furthermore, chimpanzees have been reported to infer disturbance in environments via observation of other conspecifics. For example, Menzel (1971, 1974), who studied dynamics of a group of chimpanzees from a locational perspective, conducted a study in which an object associated with fear (e.g., a plastic model of a snake or alligator) was concealed in an enclosure while one individual (“leader”) in a group of chimpanzees watched the item being hidden. When the group of chimpanzees was released into the enclosure, the members who did not see what was hidden (“followers”) also exhibited cautious behaviors, which suggested that they were influenced by the behavior of the leader individual who knew what was hidden. In addition, recent experiments suggest that playbacks of alarm calls elicit greater attention to a speaker (Crockford et al. 2015, 2017) and marking-like behaviors in conjunction with alarm calls were found to evoke a cautious approach in groupmates (Crockford et al. 2017). Moreover, a group of wild chimpanzees were speculated to have learned how to deactivate snares set by humans in the forest by observing other groupmates deactivating snares, which possibly led to the avoidance of injury (Ohashi and Matsuzawa 2011).

These findings suggest that chimpanzees might have some understanding of others' behavior in unusual conditions and others' reactions to disturbing situations. However, most of this evidence is based on observations of overt behaviors, which are often difficult to interpret. Thus, systematic investigation is needed to decipher the underlying cognitive and affective processes. In the current thesis, I sought to extend the line of inquiry regarding chimpanzees' understanding of others to their understanding of others in unusual conditions and their understanding of others' reactions to irregular events in their environment.

To achieve this aim, I focused on visual attention among chimpanzees. In foveated animal species, eye movements play a pivotal role in sampling information from surrounding environments (e.g., Gottlieb et al. 2013). I focused on chimpanzees' eye movements, which were measured via an eye tracker, when chimpanzees watched visual stimuli such as photos, animations, or videos. Measurements of looking time have long been utilized to elucidate various facets of cognitive processes in human developmental psychology and animal psychology (reviewed by Winters et al. 2015). The implementation of contact-free eye tracking has facilitated great ape cognition research for the past decade (reviewed by Kano and Call 2017; Hopper et al. 2020). Recent research on ape theory-of-mind highlighted the advantages of focusing on gaze behaviors (Kano et al. 2017): 1) this approach allows researchers to tap into a different level of cognitive processes from those that are revealed by the observation of overt behaviors; 2) it can reduce the cognitive demands required to succeed in the task (see also Yamamoto 2021 for discussion of cognitive and motivational obstacles in cognitive studies of chimpanzees); and 3) it can avoid causing apes to become too excited to demonstrate their cognitive capacities because of the presence of food rewards. Similar arguments may also be true for a wide range of social cognitive processes, including the processes investigated in the current thesis.

1.2 Research on Affective Processes Using Physiological Measurements

In the current thesis, I also sought to explore possible concurrent affective responses in chimpanzees, which are closely tied to attention (Bradley 2009). In a functionalist approach (e.g., Adolphs and Andler 2018), studying animal affective processes, as distinct from other cognitive processes, is considered to be possible by examining its possible behavioral, physiological and cognitive manifestations. In addition, the researcher remains provisionally agnostic about their concurrent conscious experience (i.e., feelings), thereby aiming to extend the current state of affective science of nonhuman animals (i.e., comparative affective science; Williams et al. 2020). In the current thesis, I sought to apply physiological measurements to chimpanzees (nasal skin temperature in *Chapter 2*, pupil dilation in *Chapter 3*) and tested an alternative possibility based on a simple affective response (*Chapter 4*).

Measurements of peripheral physiological changes have long been used to study human affective states, which involve autonomic nervous system (ANS) activity such as cardiovascular, electrodermal, and respiratory reactions (e.g., Siegel et al. 2018). Over the past several decades, various studies have used physiological techniques for examining chimpanzees, providing possible implications for their affective processes (e.g., Berntson and Boysen 1989; Berntson et al. 1989; Boysen and Berntson 1989; Parr 2001).

In one previous study, a juvenile chimpanzee was presented with photographs of either a familiar peer individual, a familiar aggressive individual, or an unfamiliar individual, while electrocardiographic measurements were taken via electrodes attached to their body

(Boysen and Berntson 1989). A greater heart-rate increase was observed when the chimpanzee viewed a photo of an aggressive individual compared with when they viewed other stimuli. In addition, a greater decrease was observed when the chimpanzee viewed a photo of an unfamiliar individual compared with when they viewed a photo of a familiar peer individual, whereas no significant difference was observed in heart-period variability (see Berntson and Boysen 1990 for an overview of their research on cardiac responses).

Parr (2001) carried out skin temperature measurement with three captive chimpanzees via temperature transducers attached to the fingertip while they viewed 6-s video clips depicting several affective scenes. Skin temperature was found to decrease, which indicated increased arousal, when chimpanzees viewed conspecifics being injected with a hypodermic needle or darted by a dart-gun, compared with when they viewed conspecifics being threatened by a human veterinarian. As described in the previous section, chimpanzees also underwent a cognitive task, in which they were found to readily learn to match videos with corresponding facial expressions in terms of valence. These findings suggest that concurrent physiological responses may modulate visual processing of affective information. These previous studies shed light on the usefulness of physiological measurements in great ape research.

However, a major difficulty in these earlier studies comes from attaching devices directly to animals’ bodies, which can be rejected by chimpanzees, and can thus only be applied with certain tolerant individuals (Kano et al. 2016). Consequently, the studies described above often involved a limited range of participants, such as nursery-reared (coupled with interactions with peers) and/or very young individuals. Contact-free methods may be helpful for overcoming this obstacle. In particular, skin temperature measurement via thermography and pupillometry are expected to be viable approaches for revealing aspects of nonhuman primate affect, which cannot be revealed by observation with the naked eyes (Kret et al. 2020; Kuraoka and Nakamura 2022).

Recent advances in infrared thermal imaging have opened up an avenue for examining affective states in animals via measurement of skin temperature in a non-invasive, contact-free way (reviewed in Kuraoka and Nakamura 2022; Sato et al. 2018; Travain and Valsecchi 2021). To date, two studies have used infrared thermography to examine arousal in chimpanzees (Kano et al. 2016; Dezecache et al. 2017). One previous study measured chimpanzees' nasal temperature using an infrared thermo-camera when presented with auditory stimuli of chimpanzees fighting, other species' calls (i.e., orangutan long call), and no sound (Kano et al. 2016, Experiment 1). A greater temperature decrease, which indicated increased arousal, was observed when chimpanzees heard conspecific fighting compared with when they heard other species' calls or no sound. It was also found that salivary cortisol did not show a substantial increase, suggesting that physiological responses were mainly based on sympathetic nervous system activity rather than hypothalamus-pituitary-adrenal axis activity. A similar temperature decrease was observed when chimpanzees viewed audio-visual stimuli of conspecific fighting (Kano et al. 2016, Experiment 2). In addition, an individual was examined by attaching three electrodes to the wrists and foot to measure heart rate. Heart rate variability (particularly the relative power of high frequency band) was found to differ when that individual viewed conspecific fighting compared with other movies (i.e., conspecifics resting or a blank screen), suggesting that parasympathetic nervous system activity was decreased when the fighting movie was viewed. Another study used infrared thermal imaging in a wild setting to examine chimpanzees' reactions toward conspecific vocalizations that occurred naturally (rather than playbacks) (Dezecache et al. 2017). Some types of vocalizations were found to elicit decreases in nasal temperature and increases in ear temperature. These previous studies highlighted the effectiveness of infrared thermography in studies of chimpanzees.

Pupillometry is another potentially useful contact-free method (e.g., Hepach and Westermann 2016; Mathôt 2018). A recent study used this technique to examine chimpanzee arousal (Hepach et al. 2021). In that study, chimpanzees were either allowed or not allowed to assist another conspecific individual (stooge) to obtain a food item by pulling a rope, which moved the food item toward a position that was accessible to the stooge (Hepach et al. 2021, Study 1). While measuring chimpanzee pupil diameter, chimpanzees were presented with a plain occlude, and a light was turned on and off to induce the pupillary reflex, which allowed the researchers to analyze the local minimum of pupil diameter. A greater change in pupil diameter was found when chimpanzees were not allowed to assist the stooge compared with when they could assist the stooge, suggesting that completion of helping behavior reduced arousal and/or that experiencing the disruption of helping increased chimpanzees' arousal. Such a differentiated pupil response was not found in the control conditions in which the stooge chimpanzee was absent. In Hepach et al.'s (2021) second experiment (Study 2), chimpanzees were either allowed or not allowed to pull the rope. However, this time, the food item was delivered to those chimpanzees themselves rather than to another individual. Changes in pupil diameter were no different regardless of whether chimpanzees obtained the food item themselves or a human experimenter gave them the food item, suggesting that the results of Study 1 were not explained by the movements the chimpanzees (i.e., pulling the rope) or the human experimenter (i.e., picking up the food) performed in the presence of another conspecific individual. Although Hepach et al. (2021) acknowledged that measuring chimpanzees' pupil diameter requires chimpanzees' eyes to be tracked stably for at least 10 s, which reduced the sample size (and other procedural details were ambiguous, including the way in which the experimenter managed to keep the chimpanzees' gaze on the plain occluder), their findings highlighted the potential usefulness of pupillometry in studying great apes (see also Krupenye et al. 2021 for a similar preliminary attempt).

Overall, contact-free physiological measurements focusing on autonomic nervous system activity (e.g., thermal imaging and pupillometry) are broadening the range of tools available for chimpanzee affective science, but these methods are still in their infancy. Thus, there is a need for more empirical data to establish reliable methodologies. In the current studies, I attempted to apply these techniques to provide further insight into chimpanzee psycho-physiology.

1.3 Aim of the Current Thesis

I sought to shed light on some of the unexplored aspects of chimpanzees' ability to understand others. Specifically, I carried out experiments on chimpanzees to investigate cognitive and affective processes when chimpanzees cope with an irregular, unusual, or emergent situation that would be likely to disturb their ordinary routine and require individuals to exercise their cognitive abilities. As irregular situations are by definition infrequent and difficult to replicate in real-life, chimpanzees' responses to irregular circumstances can be best studied in laboratory experiments, such as presenting movie stimuli in conjunction with eye tracking, which enables the examination of apes' subtle reactions to scenes that would otherwise be difficult to substantiate (Kano et al. 2017).

In *Chapter 2*, I first examined chimpanzees' visual attention toward injured conspecifics using an eye tracking experiment. Next, I examined whether chimpanzees exhibit affective responses upon observing others' injury by measuring nasal skin temperature in a thermal imaging experiment.

That research revealed cognitive and affective aspects of reactions to others' injury, which involved visual processing of a relatively static scene. In *Chapter 3*, I examined chimpanzees' understanding of biomechanics underlying dynamic movements in others, using a design that followed that of a previous eye tracking study in humans (Morita et al. 2012). Moreover, I attempted to analyze pupil diameter to examine chimpanzees' affective reactions toward impossible movements, which evoked greater arousal in the case of humans (Morita et al. 2012).

That research (*Chapters 2* and *3*) examined chimpanzees' reactions to others in unusual conditions and mainly involved visual information processing. In *Chapter 4*, I extended the inquiry into how chimpanzees react to others that are reacting to environmental events, focusing on the audio-visual cross-modal domain. Specifically, I examined whether chimpanzees spontaneously match other conspecifics' vocalizations (alarm and food-associated calls) with relevant objects (snakes and fruits, respectively). Although I did not use physiological measurements (unlike in *Chapters 2* and *3*), I tested an alternative hypothesis based on an affective response toward arousing vocalizations, thereby addressing affective processes as well as cognitive processes.

1.4 References

Adolphs R, Andler D (2018) Investigating emotions as functional states distinct from feelings. Emot Rev 10(3):191–201. https://doi.org/10.1177/1754073918765662

Adriaense JEC, Koski SE, Huber L, Lamm C (2020) Challenges in the comparative study of empathy and related phenomena in animals. Neurosci Biobehav Rev 112:62–82. https://doi.org/10.1016/j.neubiorev.2020.01.021

Amati S, Babweteera F, Wittig RM (2008) Snare removal by a chimpanzee of the Sonso community, Budongo Forest. Pan Africa News 15(1):6–8. https://doi.org/10.5134/143488

Barrett L, Henzi P, Rendall D (2007) Social brains, simple minds: does social complexity really require cognitive complexity? Philos Trans R Soc B Biol Sci 362(1480):561–575. https://doi.org/10.1098/rstb.2006.1995

Barrett L, Henzi SP, Barton RA (2022) Experts in action: why we need an embodied social brain hypothesis. Phil Trans R Soc B Biol Sci 377(1844):20200533. http://doi.org/10.1098/rstb.2020.0533

Berntson GG, Boysen ST (1989) Specificity of the cardiac response to conspecific vocalizations in chimpanzees. Behav Neurosci 103(2):235–245. https://doi.org/10.1037/0735-7044.103.2.235

Berntson GG, Boysen ST (1990) Cardiac indices of cognition in infants, children, and chimpanzees. In: Rovee-Collier C, Lipsitt LP (eds) Advances in infant research, vol 6. Ablex, Norwood, pp 187–220

Berntson GG, Boysen ST, Bauer HR, Torello MS (1989) Conspecific screams and laughter: cardiac and behavioral reactions of infant chimpanzees. Dev Psychobiol 22(8):771–787. https://doi.org/10.1002/dev.420220803

Bettle R, Rosati AG (2021) The primate origins of human social cognition. Lang Learn Dev 17(2):96–127. https://doi.org/10.1080/15475441.2020.1820339

Boysen ST, Berntson GG (1989) Conspecific recognition in the chimpanzee (*Pan troglodytes*): cardiac responses to significant others. J Comp Psychol 103(3):215–220. https://doi.org/10.1037/0735-7036.103.3.215

Bradley MM (2009) Natural selective attention: orienting and emotion. Psychophysiology 46(1):1–11. https://doi.org/10.1111/j.1469-8986.2008.00702.x

Brooker JS, Webb CE, Clay Z (in press) Primate empathy: a flexible and multi-componential phenomenon. In: Schwartz BL, Beran MJ (eds) Primate cognition studies. Cambridge University Press, Cambridge

Buttelmann D, Buttelmann F, Carpenter M, et al (2017) Great apes distinguish true from false beliefs in an interactive helping task. PLoS ONE 12(4):e0173793. https://doi.org/10.1371/journal.pone.0173793

Buttelmann D, Call J, Tomasello M (2009) Do great apes use emotional expressions to infer desires? Dev Sci 12(5):688–698. https://doi.org/10.1111/j.1467-7687.2008.00802.x

Clark IR, Sandel AA, Reddy RB, Langergraber KE (2021) A preliminary analysis of wound care and other-regarding behavior in wild chimpanzees at Ngogo, Kibale National Park, Uganda. Primates 62(5):697–702. https://doi.org/10.1007/s10329-021-00925-7

Crockford C, Wittig RM, Zuberbühler K (2017) Vocalizing in chimpanzees is influenced by social-cognitive processes. Sci Adv 3(11):e1701742. https://doi.org/10.1126/sciadv.1701742

Crockford C, Wittig RM, Zuberbühler K (2015) An intentional vocalization draws others' attention: a playback experiment with wild chimpanzees. Anim Cogn 18(3):581–591. https://doi.org/10.1007/s10071-014-0827-z

de Waal FBM, Preston SD (2017) Mammalian empathy: behavioural manifestations and neural basis. Nat Rev Neurosci 18(8):498–509. https://doi.org/10.1038/nrn.2017.72

Dezecache G, Zuberbühler K, Davila-Ross M, Dahl CD (2017) Skin temperature changes in wild chimpanzees upon hearing vocalizations of conspecifics. R Soc Open Sci 4(1):160816. https://doi.org/10.1098/rsos.160816

Dunbar RIM, Shultz S (2017) Why are there so many explanations for primate brain evolution? Phil Trans R Soc B 372(1727):20160244. http://doi.org/10.1098/rstb.2016.0244

Ferretti V, Papaleo F (2018) Understanding others: emotion recognition abilities in humans and other animals. Genes Brain Behav 18(1):e12544. https://doi.org/10.1111/gbb.12544

Freeberg TM, Gentry KE, Sieving KE, Lucas JR (2019) On understanding the nature and evolution of social cognition: a need for the study of communication. Anim Behav 155:279–286. https://doi.org/10.1016/J.ANBEHAV.2019.04.014

Goodall J (1986a) The chimpanzees of Gombe: patterns of behavior. Harvard University Press, Cambridge, pp 357–386. Japanese edition: Yukoteki kodo. In: Yasei chinpanji no sekai (trans: Sugiyama Y, Matsuzawa T). Minerva Shobo, Kyoto, pp 365–397

Goodall J (1986b) Social rejection, exclusion, and shunning among the Gombe chimpanzees. Ethol Sociobiol 7(3–4):227–236. https://doi.org/10.1016/0162-3095(86)90050-6

Gottlieb J, Oudeyer P-Y, Lopes M, Baranes A (2013) Information-seeking, curiosity, and attention: computational and neural mechanisms. Trends Cogn Sci 17(11):585–593. https://doi.org/10.1016/j.tics.2013.09.001

Hebb DO (1946) On the nature of fear. Psychol Rev 53(5):259–276. https://doi.org/10.1037/h0061690

Hepach R, Vaish A, Kano F, et al (2021) Chimpanzees' (*Pan troglodytes*) internal arousal remains elevated if they cannot themselves help a conspecific. J Comp Psychol 135(2):196–207. https://doi.org/10.1037/com0000255

Hepach R, Westermann G (2016) Pupillometry in infancy research. J Cogn Dev 17(3):359–377. https://doi.org/10.1080/15248372.2015.1135801

Hopper LM, Gulli RA, Howard LH, et al (2020) The application of noninvasive, restraint-free eye-tracking methods for use with nonhuman primates. Behav Res Methods 53(3):1003–1030. https://doi.org/10.3758/s13428-020-01465-6

Itakura S (1994) Differentiated responses to different human conditions by chimpanzees. Percept Mot Skills 79(3):1288–1290. https://doi.org/10.2466/pms.1994.79.3.1288

Kano F, Call J (2017) Great ape social attention. In: Watanabe S, Hofman MA, Shimizu T (eds) Evolution of the brain, cognition, and emotion in vertebrates. Brain science. Springer, Tokyo, pp 187–206. https://doi.org/10.1007/978-4-431-56559-8_9

Kano F, Hirata S, Deschner T, et al (2016) Nasal temperature drop in response to a playback of conspecific fights in chimpanzees: a thermo-imaging study. Physiol Behav 155:83–94. https://doi.org/10.1016/j.physbeh.2015.11.029

Kano F, Krupenye C, Hirata S, et al (2019) Great apes use self-experience to anticipate an agent's action in a false-belief test. Proc Natl Acad Sci USA 116(42):20904–20909. https://doi.org/10.1073/pnas.1910095116

Kano F, Krupenye C, Hirata S, Call J (2017) Eye tracking uncovered great apes' ability to anticipate that other individuals will act according to false beliefs. Commun Integr Biol 10(2):e1299836. https://doi.org/10.1080/19420889.2017.1299836

Kim Y, Kret M (2022) The emotional expressions and emotion perception in nonhuman primates. In: Dukes D, Samson AC, Walle EA (eds) The Oxford handbook of emotional development. Oxford University Press, Oxford, pp 129–145

Kret ME, Prochazkova E, Sterck EHM, Clay Z (2020) Emotional expressions in human and non-human great apes. Neurosci Biobehav Rev 115:378–395. https://doi.org/10.1016/j.neubiorev.2020.01.027

Krupenye C, Call J (2019) Theory of mind in animals: current and future directions. Wiley Interdiscip Rev Cogn Sci 10(6):e1503. https://doi.org/10.1002/wcs.1503

Krupenye C, Köster M, Clay Z (2021) Bonobos' (*Pan paniscus*) and chimpanzees' (*Pan troglodytes*) understanding of, and pupillary responses to, others' needs. In: Proceedings of the Annual Meeting of the Cognitive Science Society. p 3276

Kuraoka K, Nakamura K (2022) Facial temperature and pupil size as indicators of internal state in primates, Neurosci Res. https://doi.org/10.1016/j.neures.2022.01.002

Leroux M, Monday G, Chandia B, et al (2021) First observation of a chimpanzee with albinism in the wild: social interactions and subsequent infanticide. Am J Primatol:e23305. https://doi.org/10.1002/ajp.23305

Lewis L, Krupenye C (in press) Theory of mind in nonhuman primates. In: Schwartz B, Beran M (eds) Primate cognitive studies. Cambridge University Press, Cambridge.

Mathôt S (2018) Pupillometry: psychology, physiology, and function. J Cogn 1(1):1–23. https://doi.org/10.5334/joc.18

Menzel EW Jr (1971) Communication about the environment in a group of young chimpanzees. Folia Primatol 15(3–4):220–232. https://doi.org/10.1159/000155381

Menzel EW Jr (1974). A group of young chimpanzees in a one-acre field. In: Schrier AM, Stollnitz F (eds) Behavior of nonhuman primates: modern research trends, vol 5. Academic Press, New York, pp 83–153. https://doi.org/10.1016/B978-0-12-629105-6.50009-2

Morita T, Slaughter V, Katayama N, et al (2012) Infant and adult perceptions of possible and impossible body movements: an eye-tracking study. J Exp Child Psychol 113(3):401–414. https://doi.org/10.1016/j.jecp.2012.07.003

Nieuwburg EGI, Ploeger A, Kret ME (2021) Emotion recognition in nonhuman primates: How experimental research can contribute to a better understanding of underlying mechanisms. Neurosci Biobehav Rev 123:24–47. https://doi.org/10.1016/j.neubiorev.2020.11.029

Nolte S, Call J (2021) Targeted helping and cooperation in zoo-living chimpanzees and bonobos. R Soc Open Sci 8(3):201688. https://doi.org/10.1098/rsos.201688

O’Connell SM (1995) Empathy in chimpanzees: evidence for theory of mind? Primates 36(3):397–410. https://doi.org/10.1007/BF02382862

Ohashi G, Matsuzawa T (2011) Deactivation of snares by wild chimpanzees. Primates 52(1):1–5. https://doi.org/10.1007/s10329-010-0212-8

Parr LA (2001) Cognitive and physiological markers of emotional awareness in chimpanzees (*Pan troglodytes*). Anim Cogn 4(3–4):223–229. https://doi.org/10.1007/s100710100085

Sato Y, Kano F, Hirata S (2018) Cutting-edge infrared thermography as a new tool to explore animal emotions. Japanese J Anim Psychol 68(1):1–15. https://doi.org/10.2502/janip.68.1.7

Seyfarth RM, Cheney DL (2013) Affiliation, empathy, and the origins of theory of mind. Proc Natl Acad Sci USA 110(Suppl 2):10349–10356. https://doi.org/10.1073/pnas.1301223110

Siegel EH, Sands MK, Van den Noortgate W, et al (2018) Emotion fingerprints or emotion populations? A meta-analytic investigation of autonomic features of emotion categories. Psychol Bull 144(4):343–393. https://doi.org/10.1037/bul0000128

Slocombe KE, Townsend SW, Zuberbühler K (2009) Wild chimpanzees (*Pan troglodytes schweinfurthii*) distinguish between different scream types: evidence from a playback study. Anim Cogn 12(3):441–449. https://doi.org/10.1007/s10071-008-0204-x

Travain T, Valsecchi P (2021) Infrared thermography in the study of animals’ emotional responses: a critical review. Animals 11(9):2510. https://doi.org/10.3390/ani11092510

Watanabe S (2017) Empathy and common experience in animals. Jpn J Cogn Neurosci 19(3–4):109–117. https://doi.org/10.11253/ninchishinkeikagaku.19.109

Whiten A (2000) Social complexity and social intelligence. In: Bock GR, Goode JA, Webb K (eds) The nature of intelligence: Novartis Foundation Symposium 233, vol 233. Wiley, Chichester, pp 185–201

Williams LA, Brosnan SF, Clay Z (2020) Anthropomorphism in comparative affective science: advocating a mindful approach. Neurosci Biobehav Rev 115:299–307. https://doi.org/10.1016/j.neubiorev.2020.05.014

Winters S, Dubuc C, Higham JP (2015) Perspectives: the looking time experimental paradigm in studies of animal visual perception and cognition. Ethology 121(7):625–640. https://doi.org/10.1111/eth.12378

Yamamoto S (2017) Primate empathy: three factors and their combinations for empathy-related phenomena. WIREs Cogn Sci 8(3):e1431. https://doi.org/10.1002/wcs.1431

Yamamoto S (2021) “unwilling” versus “unable”: understanding chimpanzees’ restrictions in cognition and motivation. Psychologia. https://doi.org/10.2117/psysoc.2021-b020

Yamamoto S, Humle T, Tanaka M (2012) Chimpanzees’ flexible targeted helping based on an understanding of conspecifics’ goals. Proc Natl Acad Sci USA 109(9):3588–3592. https://doi.org/10.1073/pnas.1108517109

Chapter 2

Spontaneous Attention and Psycho-Physiological Responses to Others' Injury in Chimpanzees

2.1 Abstract

Previous studies have shown that humans experience negative emotions when seeing contextual cues of others' pain, such as injury (i.e., empathic pain), even without observing behavioral expressions of distress. However, this phenomenon has not been examined in nonhuman primates. We tested six chimpanzees (*Pan troglodytes*) to experimentally examine their reactions to others' injury. First, we measured viewing responses using eye-tracking. Chimpanzees spontaneously attended to injured conspecifics more than non-injured conspecifics, but did not do so in a control condition in which images of injuries were scrambled while maintaining color information. Chimpanzees did not avoid viewing injuries at any point during stimulus presentation. Second, we used thermal imaging to investigate chimpanzees' physiological responses to others' injury. Previous studies reported that reduced nasal temperature is a characteristic of arousal, particularly arousal associated with negative valence. We presented chimpanzees with a realistic injury: a familiar human experimenter with a prosthetic wound and artificial running blood. Chimpanzees exhibited a greater nasal temperature reduction in response to injury compared with the control stimulus. Finally, chimpanzees were presented with a familiar experimenter who stabbed their (fake) thumb with a needle, with no running blood, a situation that may be more challenging in terms of understanding the cause of distress. Chimpanzees did not physiologically distinguish this condition from the control condition. These results suggest that chimpanzees inspect others' injuries and become aroused by seeing injuries even without observing behavioral cues, but have difficulty doing so without explicit (or familiar) cues (i.e., open wound and blood).

Keywords: *Pan troglodytes,* Injury, Pain, Attention, Skin temperature, Emotion

2.2 Introduction

Caring for injured or weak members is common among humans. This behavior appears to have an ancient evolutionary origin in *Homo*, and fossil records indicate that Neanderthal individuals with severe injuries and disabilities survived for relatively long periods, suggesting that they routinely received care from community members (Spikins et al. 2018). Although such routine care may be less frequent in nonhuman animals (Turner et al. 2014), some species have been observed to show affiliation and offer helps to distressed conspecifics (e.g., rodents: Burkett et al. 2016; ravens: Fraser and Bugnyar 2010, wolves: Palagi and Cordoni 2009; great apes: de Waal and van Roosmalen 1979; dolphins: Warren-Smith and Dunn 2006; elephants: Plotnik and de Waal 2014). One proposed psychological mechanism underlying these behaviors is empathy for others in distress (e.g., Decety et al. 2016; Frank and Linsenmair 2017; de Waal 2008). The term "empathy" involves multi-faceted phenomena, ranging from reflexive or automatic matching with others, such as emotional contagion and behavioral mimicry, to more cognitively controlled or sympathetic behaviors, such as consolation and targeted helping (e.g., de Waal and Preston 2017; Heyes 2018; Seyfarth and Cheney 2013; Yamamoto 2017). Empathy is reported to be modulated by observers' individual traits, such as previous knowledge or experience (e.g., Sato et al. 2015), and the social relationships between the sufferers and the observers, such as familiarity and kinship (e.g., Burkett et al. 2016).

Empathetic responses to others in distress can either be based on the sufferer's behavioral expressions, such as screaming or distressed facial expressions, or contextual cues, such as injury or inability to escape from a trap (Goubert et al. 2005, 2009). A variety of species have been reported to show affiliation to injured conspecifics after fights or accidents (e.g., monkeys: Campbell et al. 2016; Clyvia et al. 2014; Dittus and Ratnayeke 1989; dolphins: Warren-Smith and Dunn 2006; elephants: Douglas-Hamilton et al. 2006).

Chimpanzees are one of the species in which this behavior has been most frequently documented. For example, chimpanzees have often been observed to inspect, groom, or lick groupmates’ injuries (Boesch 1991, 1992; Goodall 1986; Nishida and Hiraiwa-Hasegawa 1985). In addition, a group of chimpanzees was observed to wait for an injured individual who was left behind when the group traveled to another location (Boesch 1992). Moreover, an adolescent male chimpanzee was observed to help carry an infant when the mother chimpanzee was injured (Pruetz 2011). Finally, an adult male chimpanzee was observed to help remove a snare from a trapped groupmate (Amati et al. 2008; also see Tokuyama et al. 2012 for a similar behavior in bonobos).

These observations are informative because they suggest that chimpanzees respond to others’ distress not only when seeing a behavioral cue (based on a specific emotion possibly developed for species-typical communication) but also when seeing a contextual cue (based on understanding a cause of distress); both observations together provide more stringent evidence for empathy in chimpanzees. However, for the latter observations, previous studies have mainly documented behaviors rather than internal responses. Thus, it remains unclear whether chimpanzees experience negative emotions in response to others’ injuries while offering affiliation/help to them. Previous studies with human participants have reported that humans experience negative emotion (i.e., empathetic pain) when observing “painful” situations in which other individuals accidentally harm themselves or undergo injury (Goubert et al. 2005, 2009; Kupfer 2018). This line of studies has utilized a variety of physiological measures to evaluate participants’ internal responses, such as heart rate (Preis and Kroener-Herwig 2012), skin conductance (Forgiarini et al. 2011; Hein et al. 2011), pupil diameter (Azevedo et al. 2013), and skin temperature changes (Salazar-López et al. 2015).

However, these methods have rarely been used in studies of chimpanzees. As a notable exception, Parr (Parr 2001; Study 1) examined decreases in finger skin temperature as an index of physiological arousal among chimpanzees when they were observing video clips depicting conspecifics being injected with a hypodermic needle. Chimpanzees were found to exhibit a temperature decrease in response to the video clip. However, they responded similarly to a control video clip depicting a hypodermic needle alone. Thus, it remains unclear whether chimpanzees empathized with conspecifics in distress, or simply responded to the hypodermic needle as an aversive stimulus. Therefore, further studies are necessary to elucidate this issue. Such studies could provide important evidence regarding the question of whether nonhuman animals exhibit empathic responses in the absence of suffers' behavioral cues, and also could help to establish an experimental approach that is similar to the research paradigms used with human participants.

However, at least two major methodological challenges are involved in performing such studies. First, the contextual cues that elicit emotional responses in chimpanzees in a laboratory setting are currently unclear. Given that the video of a needle injection used by Parr (2001) was somewhat ambiguous in terms of the aversive value of a needle, it may be useful to conduct exploratory experiments to examine this issue. Second, it is practically challenging to measure physiological responses in large-bodied animals such as chimpanzees because untrained chimpanzees typically do not accept the use of electrodes, which are necessary for most physiological measurement techniques. However, recent advances in thermal imaging techniques provide a promising avenue for research because the technique does not require direct contact (e.g., Ioannou et al. 2014; Sato et al. 2018).

Thermal imaging has been used to measure physiological changes of an animal by observing changes in skin temperature. For example, Kuraoka and Nakamura (2011) reported that conspecific screams (behavioral cues of distress) induced changes in skin conductance and a decrease in nasal skin temperature in rhesus monkeys. Following this previous study, Kano et al. (2016) reported that conspecific screams induced changes in heart rate variability and a drop in nasal skin temperature in chimpanzees. Dezecache et al. (2017) reported similar results in a field setting. These studies provide evidence that monkeys and chimpanzees respond emotionally to behavioral cues, such as others' distress calls, but it remains unclear if they do so in response to contextual cues, such as others' injury.

Therefore, based on the previous studies discussed above, particularly Parr (2001), Kano et al. (2016), and Dezecache et al. (2017), the current study sought to experimentally investigate chimpanzees' physiological responses to others' injury using thermal imaging techniques. In addition, we used eye-tracking to examine chimpanzees' gaze behavior in response to others' injury. The purpose of the eye-tracking experiment was to confirm previous reports of chimpanzees spontaneously inspecting others' injuries (e.g., Boesch 1991; Goodall 1986; Nishida and Hiraiwa-Hasegawa 1985). If chimpanzees both visually inspect and physiologically react to others' injury, these observations together would strengthen the notion that they inspect injured others not only out of interest but also out of empathy.

2.3 Study 1

Chimpanzees have been observed to gently inspect conspecifics' injuries (e.g., Boesch 1991; Goodall 1986; Nishida and Hiraiwa-Hasegawa 1985). An interesting aspect of this behavior is that chimpanzees tend to spontaneously approach others' injuries, rather than being afraid of or avoiding them. This is an important characteristic of these responses, because it may differentiate an other-regarding affective response or sympathetic concern from a personal fear or disgust. In Study 1, we examined how chimpanzees allocated their attention when they viewed pictures of conspecifics with and without injuries. We predicted that chimpanzees would attend to the conspecifics' injuries throughout the presentation time (i.e., chimpanzees would not show avoidance at any point during the presentation). We also predicted that chimpanzees would not attend to others' injuries simply because the injury had a conspicuous color (i.e., red). We tested this prediction in a control condition.

2.3.1 Methods

Participants

Six chimpanzees (*Pan troglodytes*; five females and one male, 15.3 ± 6.0 years old, mean ± SD) living at Kumamoto Sanctuary, Wildlife Research Center, Kyoto University, participated in this study. Details of the participants are shown in Table S2.1, and are available in the Great Ape Information Network (https://shigen.nig.ac.jp/gain/index.jsp). At the time of the experiment, these six chimpanzees lived in a social group adjacent to neighboring chimpanzee groups, with visual access between groups. Four of the chimpanzees were reared by their biological mothers, while the others (Mizuki and Hatsuka) were partially reared by human caretakers (and partially by their biological mothers and conspecific peers) following rejection by their biological mothers (Idani and Hirata 2006).

Ethics statement

Chimpanzees were fed with a variety of vegetables, fruits, nuts, and monkey chow three times a day. Water was available ad libitum. The chimpanzees typically spent the daytime in three outdoor enclosures connected to each other (approximately 300 m^2 in total). The outdoor enclosures were furnished with small trees, platforms, pieces of fire hose, or hammocks, providing complex three-dimensional environments in which chimpanzees could actively exercise. Additional enrichment items (e.g., pieces of jute bags) were provided daily. The indoor enclosures also contained pieces of fire hose, hammocks, and wooden stages, where the chimpanzees could comfortably rest and sleep. Neither food nor water was deprived for the purpose of the experiments, and participation in the experiments was voluntary. As predicted, we confirmed that chimpanzees were not fearful of the images of injured conspecifics, and did not avoid the experiments. Animal husbandry and study protocols complied with the Guide for Animal Research Ethics provided by the Wildlife Research Center, Kyoto University (No. WRC-2018-KS008A).

Apparatus

Participants were invited from the outdoor enclosures into an indoor experimental booth (3.0 m × 3.0 m × 2.0 m). Eye-gaze was measured using an infrared head-free eye-tracker (Tobii TX300, Tobii Technology AB, Stockholm, Sweden). Visual stimuli were presented on a 23-inch LCD monitor with a resolution of 1280 × 768 pixels, at a viewing distance of approximately 70 cm (40° × 23°). The eye-tracker and monitor were placed outside the experimental booth. Participants in the experimental booth viewed stimuli on the monitor through a transparent 1-cm polycarbonate panel. Each day before testing, we sprayed anti-fog spray on the panel to prevent condensation from chimpanzees' breath obscuring their view and recording of their eye movement through the panel. During the test, we allowed participants to sip grape juice from a nozzle attached to the panel from a custom-made juice dispenser hanging from the ceiling. In addition, an experimenter lightly held the head of some participants. We employed these procedures to encourage chimpanzees to remain focused on the monitor throughout testing (note that the eye-tracker did not require participants' heads to be firmly fixed). In the first session, we conducted two-point automated calibrations for each participant, in which we showed a small object or movie clip on each reference point. We manually checked the accuracy of the calibration before each session and repeated the calibration whenever necessary. With these calibration procedures, we confirmed that the recorded error (the distance between the target and the recorded gaze positions) were typically within a degree (Kano et al. 2011). The calibration, presentation of stimuli, and eye-movement recordings were controlled in Tobii Studio software (version 3.4.8).

Stimuli and Procedure

The experiments were conducted each day for approximately 15 min between 11 a.m. and 1 p.m., from November to December 2017. When two chimpanzees were invited together (the two mother-offspring pairs), one chimpanzee was distracted while the other was tested.

The experiment consisted of two conditions, a test condition and a scramble condition (which served as a control condition) with a within-subject design. In the test condition, we presented eight image pairs. Each pair consisted of two images (i.e., the preferential looking paradigm). Images were 600 × 600 pixels (21° × 21°) in size, and were positioned 40 pixels apart from each other with a grey-scale background (Fig. 2.1a). One image depicted a chimpanzee with a bleeding injury either on their head, limb, or genital region, and the other image of each pair depicted a different individual of the same sex with no injury. All images depicted chimpanzees that were unfamiliar to the participants. Two of eight image pairs depicted female chimpanzees. The configuration of the scenes, such the depicted chimpanzees' body parts and body postures, was matched as closely as possible between the two images in each pair. The backgrounds were blurred. We conducted image processing using Adobe Photoshop CC 2017. In the scramble condition, we used the same eight image pairs, but with pixel-scrambled masks. Specifically, in each pair, we used the scramble-filter function in Photoshop to scramble the pixels of the rectangular areas surrounding the injury of the depicted chimpanzee on one image, and those of the corresponding size-matched areas on the other image (Fig. 2.1b). This eliminated all of the form information within the rectangular areas, but kept the color and luminance of each image. The side of presentation of the injured chimpanzee (and the corresponding injury-scrambled chimpanzee) in each pair was counterbalanced between the participants. On a trial, each image pair was presented for 6 s. At the beginning of each trial (before presenting each image pair), a small animation (illustration of a nonhuman animal) was presented at the center of the screen as an attention-getting stimulus (1.5 s). Each condition (test and scramble) contained two blocks (four blocks in total), each with 4 trials (16 trials in total). Each day, one or two blocks was presented, depending on participants' willingness to continue the experiment, which took 3 or 4 days in total. One participant (Misaki) did not view the images in one trial, and this was treated as a missing trial. The order of presentation of conditions was counterbalanced between participants. The order of presentation of each trial within a block was counterbalanced between participants.

Data analysis

Eye-movement data were filtered with a default Tobii Fixation Filter. We defined areas of interest (AOIs) for the whole image (640 × 640 pixels), which were slightly larger than the image size (600 × 600 pixels) to accommodate minor errors in the recorded gaze. We also defined AOIs for injured body parts of injured chimpanzees and corresponding body parts of uninjured chimpanzees. The size of AOIs varied across image pairs, but was consistent within an image pair, occupying 9.87 ± 9.97% (mean ± SD) of the AOIs for the whole picture (i.e., 640 × 640 pixels).

To examine chimpanzees' bias in looking at either of the two images in each pair, we calculated the proportion of looking time directed to the image depicting an injured chimpanzee. Thus, we divided the time spent looking at the injury image by the total time spent looking at the two images in each trial (presenting each image pair). A higher score corresponded to a longer time spent looking at the injury image. This score was compared between two conditions (test vs. scramble) for each participant using a paired t test. When significant differences were found, we compared the score with the chance level, 0.5, using a one-sample t test. To examine the time course of the looking bias, we divided the 6 s of presentation time of an image pair into three 2-s time-bins, and calculated the proportion of looking time to the injury image for each time-bin. We then subjected the scores to a repeated-measures analysis of variance (ANOVA) with condition (test vs. scramble) and time-bin (0–2, 2–4, and 4–6 s) as within-subject factors. All statistical tests were two-tailed and the significance level was set at $\alpha = 0.05$. As effect sizes, Cohen's d and generalized eta squared were reported for the paired *t* tests and ANOVAs, respectively. Statistical analyses were performed in R (version 3.4.2).

2.3.2 Results

Chimpanzees viewed both images of each pair for 4.73 ± 0.92 s in the test condition and 3.96 ± 1.00 s in the scramble condition, respectively (hereafter, the data are presented as mean ± SD). The looking time for both images was marginally longer in the test condition than in the scramble condition (paired *t* test: $t_5 = 2.50$, $p = 0.054$). This result was expected, because the scrambled mask covered certain attention-attracting features of images (e.g., injury, chimpanzees' face). In the test condition, chimpanzees viewed the image of the injured chimpanzees for 2.73 ± 0.69 s (during which they viewed the injury AOI for 1.29 ± 0.66 s) and those of the uninjured chimpanzee for 2.01 ± 0.43 s (during which they viewed the corresponding AOI for 0.80 ± 0.25 s). In contrast, in the scramble condition, chimpanzees viewed the images of injured chimpanzees for 2.18 ± 0.71 s (during which they viewed the scrambled-injury AOI for 0.56 ± 0.25 s) and those of uninjured chimpanzees for 1.78 ± 0.63 s (during which they viewed the corresponding AOI for 0.45 ± 0.20 s).

The proportion of time spent looking at the image of an injured chimpanzee was higher in the test condition compared with the scramble condition ($t_5 = 2.65$, $p = 0.045$, $d = 1.08$; Fig. 2.2a). In addition, the proportion of time spent looking at the injury image was above chance in the test condition, whereas it was not above chance in the scramble condition (test condition: $t_5 = 3.15$, $p = 0.025$; scramble condition: $t_5 = 1.43$, $p = 0.212$). The time-bin analysis yielded no significant interaction and no significant main effect (condition × time-bin: $F_{2,10} = 0.26$, $p = 0.775$, $\eta^2_G = 0.02$; condition: $F_{1,5} = 1.66$, $p = 0.253$, $\eta^2_G = 0.03$; time-bin: $F_{2,10} = 0.72$, $p = 0.510$, $\eta^2_G = 0.07$; Fig. 2.2b).

Regarding the other behavioral responses, chimpanzees did not show any fearful expressions (e.g., grimacing) or aversive postures (e.g., moving away from the screen) during the presentation of stimuli in either condition.

2.3.3 Discussion

Chimpanzees attended to injured conspecifics for longer than they attended to uninjured conspecifics and they did not avoid viewing injuries at any point during the stimulus presentation. In addition, we found that this phenomenon was not simply driven by the conspicuous color of the injury (i.e., red color). From the results of this experiment, we were unable to exclude the possibility that chimpanzees viewed others' injuries because of their novelty (or the relatively unfamiliar nature of injured skin compared with intact skin). These possibilities were addressed in Study 2. At minimum, however, the results of Study 1 revealed that chimpanzees exhibited a tendency to look at injured conspecifics spontaneously, rather than exhibiting fear or seeking to avoid them.

2.4 Study 2

Recent reviews concluded that empathy for pain in humans is triggered by both behavioral cues, such as sufferers' emotional expressions, and contextual cues, such as injury (Goubert et al. 2005, 2009). Following this line of research, in Study 2, we examined whether chimpanzees would be physiologically aroused when viewing others' injuries without seeing pain-related behavioral cues. We used infrared thermal imaging to measure changes in nasal skin temperature in chimpanzees. Decreases in nasal skin temperature have been observed when both human and nonhuman primates are aroused, either positively or negatively, but a stronger response tends to be observed during negative arousal (Chotard et al. 2018; Dezecache et al. 2017; Ioannou et al. 2014, 2015; Kano et al. 2016; Kuraoka and Nakamura 2011; Nakayama et al. 2005). This physiological response is likely to be caused by the activation of sympathetic nervous system activity, which subsequently triggers a constriction of arteriovenous anastomosis in the nasal area (Ioannou et al. 2014). In Study 1, we observed that chimpanzees attended more to injured than uninjured conspecifics. Thus, to control for the possibility that an increased level of attention caused stronger physiological responses to the stimuli, we attempted to equalize the looking time for the stimuli, by presenting attention-grabbing stimuli in the control conditions.

Prior to Study 2, we had conducted two unsuccessful experiments, which may have failed for several potential reasons (see online Supplementary Materials for the details). In brief, in the first experiment, we presented chimpanzees with still images of either injured conspecifics (test condition) or uninjured conspecifics (control condition), as in Study 1. However, chimpanzees did not physiologically distinguish between the two conditions. This result suggests that chimpanzees viewed injured conspecifics for longer than uninjured conspecifics simply out of interest but not out of empathy in Study 1. However, it remains unclear whether the still images were sufficiently realistic or powerful to elicit emotional responses. Therefore, in the second experiment, we presented video clips of conspecifics being injected with a hypodermic needle (test condition), as in Parr (2001), and video clips of conspecifics being touched softly by a familiar human experimenter (control condition). Again, we found no difference between conditions (except for confounding variables, see Supplementary Materials). We suspect that these negative results may be partly due to methodological factors, such as low baseline nasal temperature. We also suspect that more realistic situations are important to reveal subtle emotional reactions in chimpanzees, because presenting an image of an injury on a monitor is already a somewhat an artificial or an unfamiliar situation for chimpanzees.

In Study 2, we, therefore, presented chimpanzees with a realistic demonstration by a human experimenter, as previously employed in experiments with human children (e.g., Gill and Calkins 2003; Zahn-Waxler et al. 1992). We assumed that a real-life theatrical demonstration would provide a more familiar and thus more powerful stimulus for eliciting emotions in chimpanzees. We also assumed that a familiar human could substitute for a conspecific model in this experiment, because all of our chimpanzee participants had extensive experience interacting with humans since their youth (Idani and Hirata, 2006). We presented chimpanzees with a familiar human who accidentally cut his palm, while alone (i.e., not social aggression). As a control condition, we presented chimpanzees with a seemingly equally interesting event, during which the same experimenter accidentally made his hands dirty (with bananas) and subsequently washed them with soap.

2.4.1 Methods

Participants

The same group of chimpanzees from Study 1 also participated in Study 2.

Apparatus

Participants were invited from the outdoor enclosures to an indoor sleeping room (7.5 m × 3.0 m × 3.5 m) equipped with metal mesh (instead of polycarbonate panels, because infrared light is reflected by polycarbonate panels). Nasal temperature was non-invasively measured with an infrared thermo-camera (T650sc, FLIR Systems Japan K.K., Tokyo, Japan), with a resolution of 640 × 480 pixels and a frame rate of 30 Hz (Fig. 2.3a). The emissivity was set at the default value of 0.95. The thermo-camera was installed on a tripod and positioned approximately 1 m from the metal mesh. The thermo-camera was aimed at the participant chimpanzee's face, and the angle and position of the camera were adjusted carefully in real time according to the participant's postural changes. According to the manufacturers' recommendations, the thermo-camera was switched on a minimum of 15 min before the start of the experiment. A video camera was set in front of the participant to record their looking behavior.

Stimuli

Study 2 involved two conditions. A familiar experimenter (YS), who had been working with the participants for approximately 1 year, presented chimpanzees with two types of demonstration: artificial wound and hand-washing in the test and the control conditions, respectively. In the test condition, the participant was exposed to an experimenter with a prosthetic wound and artificial blood on his right palm. The prosthetic wound was created from skin-colored wax painted with reddish eye-shadow and artificial blood (Fig. 2.4a). In addition, a tube was hidden under the right sleeve, through which fake blood was pumped over the wound. At the beginning of the demonstration, the experimenter picked up a banana and attempted to cut it with a knife (with the prosthetic wound hidden in his hand). He slipped with the knife, appearing to cut his right palm, put the banana and knife down, showed the wound to the participant chimpanzee, and let the fake blood run by quickly removing the stopper from the tube. The experimenter then used tissues to wipe the blood from the wound, and maintained this action until 180 s had passed since the beginning of the demonstration. The experimenter's gaze was kept on the wound throughout the demonstration. After the demonstration, the experimenter swiftly exited the area so that he was no longer visible to the participants. It should be noted that the experimenter did not show any behavioral signs of pain (e.g., facial/bodily expression or vocalization) during and after the demonstration. In the control condition, the experimenter performed the same actions as in the test condition, but cut the banana with the knife (instead of slipping and cutting his palm) during which he made his hands dirty with the banana, and put the banana and knife down. He then put soap on his left palm from a soap dispenser placed on the floor, rubbing his hands, and picked up a plastic bottle filled with water placed on the floor to rinse his hands (both items were also present on the floor in the test condition). The experimenter then rubbed his hands, rinsed his hands with water from a plastic bottle, put his wrist-watch back on, and used tissues to wipe the water from his hands and watch. He maintained this action until 180 s had passed since the beginning of the demonstration, then swiftly exited the area.

Procedures

The general procedures followed those described by Kano et al. (2016). We conducted testing between 11:30 a.m. and 1:30 p.m. in May and June 2018. Chimpanzees participated in one trial per testing day and two trials for each condition, a total four trials. The room temperature and humidity (mean ± SD) at the time of testing were 23.6 ± 0.7°C and 69.8 ± 8.8%, respectively. The two mother-offspring pairs were invited to the test area together, and the remaining two participants were invited alone. The mother and offspring in each pair sat next to each other and viewed the same stimuli together. This was due to the difficulty distracting one chimpanzee while showing interesting stimuli to the other. However, we confirmed that the results of chimpanzees which participated as a pair did not differ from the results of other chimpanzees, and therefore, this factor was not considered further in the main analyses.

Participants sat in front of the metal mesh (each grid was 5 cm × 5 cm in size) so that we could measure their nasal temperature through the openings of the grid. According to recommendations by Ioannou et al. (2014) and Kano et al. (2016), we employed an acclimatization period: after the entrance to the sleeping room from the outdoor compound, the chimpanzees sat still for approximately 1–5 min until the nasal temperature became stable (the temperature typically increased sharply at the beginning). During this acclimatization period, an experimenter gave the chimpanzees small pieces of food (peanuts or grapes), and another experimenter checked changes in nasal temperature visually on the monitor of the thermo-camera. We avoided giving a large amount of food as gastrointestinal activity can affect peripheral skin temperature (van Baak 2008; Kano et al. 2016). We then started the test recordings, which consisted of a stimulus presentation period (180 s), plus the preceding 3-

min and subsequent 3-min recording periods (to accommodate the 180-s post-stimulus period). Kano et al. (2016) reported that chimpanzees' nasal temperature dropped immediately after stimulus onset, continued to drop for up to 2 min, and recovered to baseline within 1 min after stimulus offset. To encourage participants to stay in front of the thermo-camera, we allowed them to sip tepid juice (grape or apple) diluted with roughly the same amount of water throughout the trial. We used identical juice dispensers to those used in Study 1. Chimpanzees could sip the juice approximately at 0.5–1.5 ml/s. Thus, chimpanzees were not distracted by the presentation of small pieces of food during the demonstration (Sato et al. 2018). When a participant walked away from the juice dispenser during stimulus presentation, we called their name to encourage them to come back, and later coded these events from the video recordings. This occurred in three trials for one male chimpanzee (Zamba; first trial of test condition: 31.3 s; first trial of control condition: 28.8 s; second trial of test condition: 8.3 s) and one trial for a female chimpanzee, albeit only briefly (Misaki; first trial of test condition: 4.9 s). Kano et al. (2016) reported that nasal temperature decreased to a greater extent as chimpanzees walked for a longer period. This effect was not considered in the current study because walking was relatively infrequent, and the duration of walking (which approximately equaled our measure of the time for which the individual left the juice dispenser) did not differ substantially between conditions.

Data Analysis

We used Research IR thermal-image analysis software to analyze the temperature data. We defined the AOI as a circle approximately 1 cm in size (measured as the relative size compared with the 5 × 5 cm grid) on the participant's nasal tip, and extracted the average temperature of the AOI every 20 s (Fig. 2.3b). In accord with the recommendation by Kano et al. (2016), each recording time point accommodated ± 5 s to avoid inappropriate frames in which the participant's face quickly moved (thereby causing a motion blur) or was angled at more than 45°. The missing values comprised 2.3% of the whole data set, and were linearly interpolated in the analysis. We assessed the inter-coder reliability of temperature extraction: another coder who was blind to the condition coded one trial for each participant (for the whole stimulus period and the first 2-min of post-stimulus period, resulting in 96 data points) and we computed the intraclass correlation coefficient based on a single rater, with an absolute-agreement, two-way random-effects model (Koo and Li 2016) using the R package, irr. This analysis yielded an acceptable level of reliability, 0.87 (95% CI = [0.02, 0.96]). The baseline was set at the onset of the demonstration. The demonstration lasted for 180 s. We also included in the analysis the following 180 s as the post-demonstration period, in which the temperature was expected to recover. We subtracted the baseline value from the following 360 s; 18 data points (i.e., 20, 40, …, 360 s from the stimulus onset).

Statistical analyses were based on linear mixed-effect models (LMMs) to analyze the time-series data. For the response variable, temperature data (the changes from baseline) were used. For the explanatory variables, we used condition (test vs. control), trial (first vs. second), time, and the square of time, time^2, as well as interactions between condition, trial, and time, and interactions between condition, trial, and time^2. We included time^2 into the model because we expected a recovery in temperature after the offset of the demonstration. We also included the random effects of participants, including both the random intercept and random slopes for each variable. However, the random slope for the three-way interaction was dropped in the final model because the model failed to converge with it.

Using the video recordings (30 Hz), chimpanzees' looking behaviors during the demonstration were coded offline. During the 180-s demonstration, participants' looking at the stimulus was defined when participants looked at either the demonstrator's hand or the blood on the floor. We also coded instances in which chimpanzees moved away from the juice dispenser, as noted earlier. Additionally, within a certain time frame, looking could not be coded because the eyes were obstructed by the metal grids or the demonstrator's body or hand. In the test condition, this occurred for 11.73 ± 12.72% in the first trial and for 8.17 ± 9.37% in the second trial, respectively (mean ± SD). In the control condition, this occurred for 27.97 ± 18.79% in the first trial and for 20.91 ± 26.37% in the second trial, respectively (mean ± SD). Thus, the items we coded included: (1) looking at stimulus, (2) leaving juice dispenser, and (3) not clearly seen (Table S2.2). Behavioral coding was performed using BORIS software (version 6.3.7 and 7.4: Friard and Gamba 2016). In the software, we replayed the video at a slower speed than the normal speed, and coded the onset/offset of each behavior using a key press. We then calculated the proportion of looking time in relation to the total observation time: [(1) / (180 s – (2) – (3))]. We compared the proportion of looking duration between conditions using a repeated-measures ANOVA with trial (first vs. second) and condition (test vs. control) as within-subject factors.

2.4.2 Results

Chimpanzees exhibited a greater decrease in nasal temperature in the test compared with the control condition (see Fig. 2.5 for the mean of each trial, and Fig. 2.6 for the individual data and the model fits). The model revealed significant main effects of condition ($\beta \pm SE = 0.33 \pm 0.12$, $df = 9.40$, $t = 2.81$, $p = 0.019$), time ($\beta \pm SE = -0.14 \pm 0.02$, $df = 19.70$, $t = -8.63$, $p < 0.001$), and time^2 ($\beta \pm SE = 0.00 \pm 0.00$, $df = 13.90$, $t = 4.72$, $p < 0.001$). The main effect of trial was not significant. We found no significant interaction effects (Table S2.3).

Regarding the looking data, no significant effect was found (condition × trial: $F_{1,5} = 0.85$, $p = 0.399$, $\eta^2_G = 0.07$; condition: $F_{1,5} = 1.18$, $p = 0.326$, $\eta^2_G = 0.06$; trial: $F_{1,5} = 1.14$, $p = 0.334$, $\eta^2_G = 0.06$), indicating that chimpanzees' attention was attracted to the demonstration to a similar degree across conditions (Fig. S2.1a).

For the other behaviors, we observed that the three adult females and one adult male interrupted their juice sipping and looked at the injury for several seconds to several tens of seconds at the beginning of the demonstration. We did not observe other identifiable behaviors, including facial and vocal expressions, in those chimpanzees.

2.4.3 Discussion

The results suggest that chimpanzees were aroused by seeing a familiar human injuring themselves. Importantly, the human demonstrator did not present any behavioral signs of pain, and chimpanzees viewed the demonstration for similar durations in both conditions. Thus, although the visual novelty could potentially have explained the observed responses of chimpanzees in Study 1, this was not the case in Study 2. Regarding behavioral responses, we did not observe any conspicuous behavioral signs of fear or distress in chimpanzees. However, we observed that three adult females and one adult male interrupted their juice sipping at the beginning of the demonstration, suggesting that they were prepared to incur at least some cost to inspect the injury.

The finding that chimpanzees were physiologically aroused while not showing any clear emotional expression suggests that the injury event was only moderately arousing. This finding also suggests that chimpanzees can be physiologically aroused without expressing emotion explicitly. This means that the absence of conspicuous emotional expression in chimpanzees does not necessarily indicate the absence of arousal, highlighting the importance of physiological measurement in experiments.

Somewhat unexpectedly, chimpanzees exhibited decreased nasal temperature in both the test and control conditions, although the response was stronger in the test condition. This result may suggest that the stimulus presented in both conditions was arousing for chimpanzees to some extent. Importantly, we suspect that procedural factors may have also been involved. For example, it is possible that giving a sip of juice during the demonstration (to encourage chimpanzees to remain in front of the thermo-camera) caused a decrease in nasal temperature. It has been previously reported that gastrointestinal activity can cause a drop in nasal temperature due to activation of the sympathetic nervous system at the initial stages of ingestion (van Baak 2008; Kano et al. 2016). Unfortunately, we were unable to exclude this potential confound in the current experiment. Kano et al. (2016) observed this phenomenon when chimpanzees ate a large amount of food at once (e.g., a whole banana) but not when they ate small pieces of food periodically (e.g., one small cut piece of grape per 10 s). Therefore, future studies should use the latter option (giving small pieces of fruit), instead of giving juice, to avoid this potential confound. In the current experiment, most importantly, we confirmed that chimpanzees exhibited a greater decrease in nasal skin temperature in the test compared with the control condition. It should be noted that three adult females ceased juice sipping when they saw the injury. However, such interruptions in juice sipping would be expected to have no effect on the nasal temperature, and therefore, do not explain the larger temperature decrease in the test condition.

Although out sample size was not sufficient to formally analyze individual differences, the results suggested potential individual differences, with three adult females exhibiting clearer results than the other chimpanzees. As previous reviews have discussed, both direct and indirect experiences of pain can strengthen the response toward others' pain in humans (e.g., Goubert et al., 2005, 2009, 2011). Likewise, it is possible that previous experiences of being injured or witnessing injured conspecifics affected the results. Future studies should address this possibility with a larger number of chimpanzees.

2.5 Study 3

Previous studies have suggested that empathy involves at least two types of processes: automatic, reflexive, and bottom-up processes; and more cognitively-controlled, top-down processes (Heyes 2018; de Waal and Preston 2017; Yamamoto 2017). Empathizing with others based on contextual cues presumably requires both processes; that is, to empathize with others' pain based on the observation of contextual cues, the observer needs to understand the cause of pain. In Study 2, we presented chimpanzees with an injury involving an open wound and running blood. Although chimpanzees were given only contextual cues but not behavioral cues (e.g., emotional expressions) in Study 2, the cognitive demand involved in understanding the cause of pain must have been relatively low because chimpanzees experience such injuries regularly in fights with groupmates. In many previous related studies with humans, the stimulus was a needle poking or penetrating a part of the body (e.g., Azebedo et al. 2013). The cognitive demand involved in understanding the cause of pain may be relatively high in this situation because needle penetration is only indicative of pain because of the nature of the needle and the body. Participants in these previous studies may have needed to infer the cause of pain based on inference from similar experiences (e.g., an injection with a hypodermic needle). In Study 3, therefore, we tested whether chimpanzees would show a similar physiological response to that observed in Study 2, even when we eliminated explicit cues such as an open wound, or blood.

2.5.1 Methods

The general procedures were identical to those of Study 2 except for the demonstrations/stimuli. The room temperature and humidity (mean ± SD) were 25.6 ± 1.0 °C and 80.8 ± 8.8%, respectively. The missing values for nasal temperature comprised 2.5% of the whole data set, and were linearly interpolated in the analysis.

For this demonstration, a familiar experimenter (FK), who had been working with the participants for longer than 3 years, acted out either stabbing a fake thumb with a needle in the test condition, or stabbing a piece of wood with a needle in the control condition. In the test condition, the experimenter was wearing a fake thumb made of plastic and painted with skin-colored powder (Fig. 2.4b). The experimenter wore fingerless gloves on both hands to hide the joints between the skin and the fake thumb. At the beginning of the demonstration, the experimenter picked up a piece of wood and attempted to stab it with a fat needle (15 cm in length and 0.3 cm in diameter) but slipped and stabbed his thumb. He then wiggled the needle and finally removed it from his thumb. The experimenter repeated this action twice and stopped the second demonstration when 180 s had passed since the beginning of the demonstration. He then swiftly left the area, so that he was no longer visible to the participants. It should be noted that, as in Study 2, the experimenter did not show any behavioral signs of pain during and after the demonstration. In the control condition, the experimenter started performing the same actions as in the test condition, but stabbed the piece of wood (instead of slipping and stabbing his thumb). He then wiggled the needle and finally removed it from the piece of wood. He repeated these actions twice, stopped the second demonstration when 180 s had passed since the beginning of the demonstration, and then swiftly left the area.

As in Study 2, we coded the looking behaviors from the video recordings. We were unable to code looking behavior in some of the recording period because of obstructions; in the test condition, this occurred for 13.64 ± 16.92% of the first trial, and for 6.02 ± 5.71% of the second trial, respectively (mean ± SD). In the control condition, this occurred for 3.23 ± 2.92% of the first trial and for 3.76 ± 3.46% of the second trial, respectively (mean ± SD). For the statistical analysis of temperature data, we used the same model as in Study 2. The random slope of the three-way interaction was dropped from the model because the model failed to converge with it.

2.5.2 Results

Chimpanzees did not exhibit changes in nasal temperature over time in any of the trials/conditions (see Fig. 2.7 for the mean of each trial, and Fig. 2.8 for the individual data and the model fits). The LMM did not reveal any significant effects (Table S2.4).

For the looking data, no significant effect was found (condition × trial: $F_{1,5} = 3.98$, $p = 0.103$, $\eta^2_G = 0.06$; condition: $F_{1,5} = 2.21$, $p = 0.198$, $\eta^2_G = 0.22$; trial: $F_{1,5} = 5.08$, $p = 0.074$, $\eta^2_G = 0.22$), indicating that their attention was attracted to the demonstration to a similar degree across conditions (Fig. S2.1b).

For the other behavior, we observed that two adult females (Misaki and Mizuki) squinted their eyes at the very moment the needle penetrated the finger in the test condition, but not in the control condition.

2.5.3 Discussion

Chimpanzees did not physiologically distinguish between the conditions in which a needle penetrated a familiar experimenter's finger or an inanimate object. This result may indicate that chimpanzees had difficulty in understanding others' pain without seeing familiar cues, such as a wound and blood. An alternative but related possibility is that direct experience is important to elicit empathic responses, as discussed in previous reviews (e.g., Goubert et al., 2005, 2009). Further studies will be needed to clarify this issue by adopting more stringent control of experiences.

It is also possible that chimpanzees exhibited subtle responses that could not be captured by the thermal imaging technique. This speculation is based on the observation that a female adult (Mizuki) exhibited reduced nasal temperature at the very moment the needle penetrated the finger (and recovered immediately after). Note that we did not expect such a brief change, and thus our model did not fit the observed decrease (Fig. 2.8). To capture such a subtle and short change, a measure of skin conductance may be more appropriate. Thus, future tests should consider the use of this technique. However, skin conductance measures should be used complementarily with thermal imaging because both techniques have strengths and limitations (c.f., Kuraoka and Nakamura 2011).

2.6 General Discussion

In previous studies, chimpanzees have been observed to spontaneously show affiliation and offer help to injured conspecifics (e.g., Boesch 1991; Goodall 1986; Nishida and Hiraiwa-Hasegawa 1985). Some researchers considered such behaviors as manifestations of empathy (e.g., de Waal 2008). The current study adds to these previous observations by showing that chimpanzees attend to injured conspecifics more than uninjured conspecifics (Study 1) and that they exhibit physiological arousal when seeing a familiar experimenter's injury with an open wound and running blood (Study 2). Importantly, we controlled for the conspicuousness of the images (i.e., the redness of the injury) in Study 1, excluding the possibility that chimpanzees only attended to conspicuous cues. We then controlled for the level of attention to the events in Study 2, suggesting that an additional psychological factor caused chimpanzees' physiological responses. In Study 3, no chimpanzee showed a similar physiological arousal when seeing a familiar experimenter stubbing his finger with a needle (without an open wound or blood), suggesting that explicit or familiar cues are necessary to elicit chimpanzees' physiological responses. Taken together, these results suggest that chimpanzees inspect others' injuries not only out of interests, but also out of empathy—at least in some familiar situations, as in Study 2 (but not in an unfamiliar situation, as in Study 3).

It could be argued that the chimpanzees may have been scared of, or disgusted with, an injury, rather than empathizing with the injured individual. However, taking the results of all experiments together, the current findings do not support this possibility. In Study 1, chimpanzees spontaneously attended to injuries rather than avoiding them, and we did not observe any conspicuous behavioral signs of distress (e.g., a fear grimace) throughout our experiments. In previous studies with humans, negative responses to images of injury are sometimes interpreted as an expression of disgust (e.g., Calvo and Lang 2004; Shenhav and Mendes 2014). However, it should be noted that the stimuli used in such studies are typically graphic, and some researchers have intentionally dissociated them from the images of “painful-injury” when selecting the stimuli (Shenhav and Mendes 2014). The stimuli in Study 2 were depictions of an injury that was similar to the injuries that chimpanzees occasionally observe after fights with groupmates. A recent study with human participants reported that people distinguish images of injuries from those of pathogen-related items, and that the images of injuries tended to be associated with a feeling of empathic pain rather than disgust (Kupfer 2018).

It could also be argued that chimpanzees might have associated the presence of injury with aggression based on past experiences, and consequently exhibited excitement to the potential initiation of a fight rather than empathy for the injured experimenter in Study 2. This explanation may be consistent with the results of Study 3, which showed that chimpanzees did not physiologically respond to the injury when neither an open wound nor blood was present. However, this interpretation cannot explain chimpanzees’ behavioral responses toward others’ injuries in the previous observations and in our experiments. In particular, in previous observations, chimpanzees have been observed to gently inspect conspecifics’ injuries, and in the current experiments, chimpanzees did not exhibit the kind of excitement that is typically displayed when witnessing fighting (e.g., body swaying, emitting pant-hoots or alarm calls; c.f., Kano et al. 2016). However, to completely rule out this possibility, it will be necessary to further explore what contextual cues could cause physiological arousal in the absence of explicit cues, such as blood.

The current findings make two key contributions to previous research, one theoretical, and the other methodological. First, previous studies have tended to focus on the behaviors rather than internal states when reporting chimpanzees’ responses to others’ injuries. The current findings revealed that chimpanzees exhibited physiological arousal when seeing others’ injuries. This finding corroborates the idea that chimpanzees can empathize with distressed others, even without behavioral cues such as emotional expressions, which may motivate chimpanzees to inspect injured groupmates.

Second, we provided a new method for studying animal empathy using a cutting-edge thermal imaging technique in combination with a real-life theatrical demonstration of an accident. Thermal imaging can provide an effective approach for studying animal emotion experimentally, particularly with large-bodied animals such as great apes (Chotard et al. 2018; Kano et al. 2016). Although our main aim was to study the effect of injury (but not behavioral cues) on chimpanzees' responses, the same method can be applied to situations involving behavioral cues in future studies. Moreover, our method showed that a theatrical demonstration of an accident is effective in experiments with chimpanzees, as in previous experiments with human children (e.g., Gill and Calkins 2003; Zahn-Waxler et al. 1992). However, it should be noted that real-life demonstration may be necessary for testing chimpanzees because our initial attempts using still images or video footage did not elicit physiological reactions to the stimuli among chimpanzees. This could be partly due to artefacts (see Supplementary Materials) but could also have occurred because the visual representation of injury was not sufficiently powerful to elicit emotion in chimpanzees.

One weakness of the current study is that the number of chimpanzees tested was relatively small, making it difficult to identify individual differences in chimpanzees' responses to others' injury. Further studies are thus necessary to test a larger number of chimpanzees using the same methods. Such studies could be useful for revealing the structure of individual differences in the observed responses, and could extend understanding of their cognitive and emotional underpinnings. Based on the current results, individual traits that should be considered in future studies include sex, age, relationship quality between the demonstrator and the observer, past experiences with the object causing pain, and a cognitive ability to understand the cause of pain. Other potential factors of interest include social competence

(Webb, Romero, Franks, and de Waal 2017) and emotional regulation (Clay and de Waal 2013), which are thought to be related to consolation behavior in great apes.

In conclusion, the current findings revealed that chimpanzees spontaneously attended to conspecifics' injuries and were physiologically aroused when seeing an injured human experimenter. These findings are consistent with the notion that chimpanzees have the capacity to empathize with the distress of an injured individual. However, individual differences and the underlying cognitive mechanisms should be further explored to elucidate the precise nature of this response in future studies.

2.7 Acknowledgments

We are grateful to Dr. N. Morimura and Dr. R. Song for help conducting the experiments. We give special thanks to the staff at Kumamoto Sanctuary, particularly Dr. T. Udono, Mr. M. Teramoto and Ms. E. Nogami, for help conducting the experiments and sharing the image materials. We thank Mr. A. Itahara for help analyzing the data. We also thank Dr. T. Matsuzawa for financial support. This study was financially supported by the Ministry of Education, Culture, Sports, Science (K-CONNEX to FK), Japan Society for Promotion of Science KAKENHI (16K21108, 18H05072) to FK, (26245069, 18H05524) to SH, and (16H06283, LDG-U04) to TM, and Great Ape Information Network. We thank Benjamin Knight, MSc., from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript. We appreciate the feedbacks provided by the editor and two reviewers.

2.8 References

Amati S, Babweteera F, Wittig RM (2008) Snare removal by a chimpanzee of the Sonso community, Budongo Forest (Uganda). Pan Afr New 15(1):6–8. https://doi.org/10.5134/143488

Azevedo RT, Macaluso E, Avenanti A, Santangelo V, Cazzato V, Aglioti SM (2013) Their pain is not our pain: brain and autonomic correlates of empathic resonance with the pain of same and different race individuals. Hum Brain Mapp 34(12):3168–3181. https://doi.org/10.1002/hbm.22133

Boesch C (1991) The effects of leopard predation on grouping patterns in forest chimpanzees. Behaviour 117(3–4):220–241. https://doi.org/10.1163/156853991X00544

Boesch C (1992) New elements of a theory of mind in wild chimpanzees. Behav Brain Sci 15(1):149–150. https://doi.org/10.1017/S0140525X00067959

Burkett JP, Andari E, Johnson ZV, Curry DC, de Waal FBM, Young LJ (2016) Oxytocin-dependent consolation behavior in rodents. Science 351(6271):375–378. https://doi.org/10.1126/science.aac4785

Calvo MG, Lang PJ (2004) Gaze patterns when looking at emotional pictures: motivationally biased attention. Motiv Emot 28(3):221–243. https://doi.org/10.1023/B:MOEM.0000040153.26156.ed

Campbell LAD, Tkaczynski PJ, Mouna M, Qarro M, Waterman J, Majolo B (2016) Behavioral responses to injury and death in wild barbary macaques (*Macaca sylvanus*). Primates 57(3):309–315. https://doi.org/10.1007/s10329-016-0540-4

Chotard H, Ioannou S, Davila-Ross M (2018) Infrared thermal imaging: positive and negative emotions modify the skin temperatures of monkey and ape faces. Am J Primatol 80(5):e22863. https://doi.org/10.1002/ajp.22863

Clay Z, de Waal FBM (2013) Development of socio-emotional competence in bonobos. Proc Natl Acad Sci USA 110(45):18121–18126. https://doi.org/10.1073/pnas.1316449110

Clyvia A, Kaizer MC, Santos RV, Young RJ, Cäsar C (2014) Do wild titi monkeys show empathy? Primate Biol 1(1):23–28. https://doi.org/10.5194/pb-1-23-2014

de Waal FBM (2008). Putting the altruism back into altruism: the evolution of empathy. Annu Rev Psychol: 59(1):279–300. https://doi.org/10.1146/annurev.psych.59.103006.093625 de Waal FBM, Preston SD (2017) Mammalian empathy: behavioural manifestations and neural basis. Nat Rev Neurosci 18(8):498–509. https://doi.org/10.1038/nrn.2017.72

de Waal FBM, van Roosmalen A (1979) Reconciliation and consolation among chimpanzees. Behav Ecol Sociobiol 5(1):55–66. https://doi.org/10.1007/BF00302695

Decety J, Bartal IBA, Uzefovsky F, Knafo-Noam A (2016) Empathy as a driver of prosocial behaviour: highly conserved neurobehavioural mechanisms across species. Philos Trans R Soc B Biol Sci 371(1686):20150077. https://doi.org/10.1098/rstb.2015.0077

Dezecache G, Zuberbühler K, Davila-Ross M, Dahl CD (2017) Skin temperature changes in wild chimpanzees upon hearing vocalizations of conspecifics. R Soc Open Sci 4(1):160816. https://doi.org/10.1098/rsos.160816

Dittus WPJ, Ratnayeke SM (1989) Individual and social behavioral responses to injury in wild toque macaques (*Macaca Sinica*). Int J Primatol 10(3):215–234. https://doi.org/10.1007/BF02735201

Douglas-Hamilton I, Bhalla S, Wittemyer G, Vollrath F (2006) Behavioural reactions of elephants towards a dying and deceased matriarch. Appl Anim Behav Sci 100(1–2):87–102. https://doi.org/10.1016/j.applanim.2006.04.014

Forgiarini M, Gallucci M, Maravita A (2011) Racism and the empathy for pain on our skin. Front Psychol 2:108. https://doi.org/10.3389/fpsyg.2011.00108

Frank ET, Linsenmair KE (2017) Saving the injured: evolution and mechanisms. Commun Integr Biol 10(5–6):e1602187. https://doi.org/10.1080/19420889.2017.1356516

Fraser ON, Bugnyar T (2010) Do ravens show consolation? Responses to distressed others. PLoS ONE 5(5):e10605. https://doi.org/10.1371/journal.pone.0010605

Friard O, Gamba M (2016) BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol Evol 7(11):1325–1330. https://doi.org/10.1111/2041-210X.12584

Gill KL, Calkins SD (2003) Do aggressive/destructive toddlers lack concern for others? Behavioral and physiological indicators of empathic responding in 2-year-old children. Dev Psychopathol 15(1):55–71. https://doi.org/10.1017/S095457940300004X

Goodall J (1986) Friendly behavior. In: The chimpanzees of Gombe: patterns of behavior. Harvard University Press, Cambridge, pp 357–386. Japanese edition: Yukoteki kodo. In: Yasei chinpanji no sekai (trans: Sugiyama Y, Matsuzawa T). Minerva Shobo, Kyoto, pp 365–397

Goubert L, Craig KD, Vervoort T et al (2005) Facing others in pain: the effects of empathy. Pain 118(3):285–288. https://doi.org/10.1016/j.pain.2005.10.025

Goubert L, Craig KD, Buysse A (2009) Perceiving others in pain: experimental and clinical evidence on the role of empathy. In: Decety J, Ickes W (eds) The social neuroscience of empathy. MIT press, Cambridge, pp 153–165

Goubert L, Vlaeyen JWS, Crombez G, Craig KD (2011) Learning about pain from others: an observational learning account. J Pain 12(2):167–174. https://doi.org/10.1016/j.jpain.2010.10.001

Hein G, Lamm C, Brodbeck C, Singer T (2011) Skin conductance response to the pain of others predicts later costly helping. PLoS ONE 6(8):e22759. https://doi.org/10.1371/journal.pone.0022759

Heyes C (2018) Empathy is not in our genes. Neurosci Biobehav Rev 95(10):499–507. https://doi.org/10.1016/j.neubiorev.2018.11.001

Idani G, Hirata S (2006) Studies at the Great Ape Research Institute. In: Washburn DA (ed) Primate perspectives on behavior and cognition. American Psychological Association, Washington, pp 29–36

Ioannou S, Gallese V, Merla A (2014) Thermal infrared imaging in psychophysiology: potentialities and limits. Psychophysiology 51(10):951–963. https://doi.org/10.1111/psyp.12243

Ioannou S, Chotard H, Davila-Ross M (2015) No strings attached: physiological monitoring of rhesus monkeys (*Macaca mulatta*) with thermal imaging. Front Behav Neurosci 9(160):1–10. https://doi.org/10.3389/fnbeh.2015.00160

Kano F, Hirata S, Call J, Tomonaga M (2011) The visual strategy specific to humans among hominids: a study using the gap-overlap paradigm. Vision Res 51(23):2348–2355. https:/doi.org/10.1016/j.visres.2011.09.006

Kano F, Hirata S, Deschner T, Behringer V, Call J (2016) Nasal temperature drop in response to a playback of conspecific fights in chimpanzees: a thermo-imaging study. Physiol Behav 155:83–94. https://doi.org/10.1016/j.physbeh.2015.11.029

Koo TK, Li MY (2016) A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med 15(2):155–163. https://doi.org/10.1016/j.jcm.2016.02.012

Kupfer TR (2018) Why are injuries disgusting? Comparing pathogen avoidance and empathy accounts. Emotion 18(7):959–970. https://doi.org/10.1037/emo0000395

Kuraoka K, Nakamura K (2011) The use of nasal skin temperature measurements in studying emotion in macaque monkeys. Physiol Behav 102(3–4):347–355. https://doi.org/10.1016/j.physbeh.2010.11.029

Nakayama K, Goto S, Kuraoka K, Nakamura K (2005) Decrease in nasal temperature of rhesus monkeys (*Macaca mulatta*) in negative emotional state. Physiol Behav 84(5):783–790. https://doi.org/10.1016/j.physbeh.2005.03.009

Nishida T, Hiraiwa-Hasegawa M (1985) Responses to a stranger mother-son pair in the wild chimpanzee: a case report. Primates 26(1):1–13. https://doi.org/10.1007/BF02389043

Palagi E, Cordoni G (2009) Postconflict third-party affiliation in *Canis lupus*: do wolves share similarities with the great apes? Anim Behav 78(4):979–986. https://doi.org/10.1016/j.anbehav.2009.07.017

Parr LA (2001) Cognitive and physiological markers of emotional awareness in chimpanzees (*Pan troglodytes*). Anim Cogn 4(3–4):223–229. https://doi.org/10.1007/s100710100085

Plotnik JM, de Waal FBM (2014) Asian elephants (*Elephas maximus*) reassure others in distress. PeerJ 2:e278. https://doi.org/10.7717/peerj.278

Preis MA, Kroener-Herwig B (2012) Empathy for pain: the effects of prior experience and sex. Eur J Pain 16(9):1311–1319. https://doi.org/10.1002/j.1532-2149.2012.00119.x

Pruetz JD (2011) Targeted helping by a wild adolescent male chimpanzee (*Pan troglodytes verus*): evidence for empathy? J Ethol 29(2):365–368. https://doi.org/10.1007/s10164-010-0244-y

Salazar-López E, Domínguez E, Ramos VJ et al (2015) The mental and subjective skin: emotion, empathy, feelings and thermography. Conscious Cogn 34:149–162. https://doi.org/10.1016/j.concog.2015.04.003

Sato N, Tan L, Tate K, Okada M (2015) Rats demonstrate helping behavior toward a soaked conspecific. Anim Cogn 18(5):1039–1047. https://doi.org/10.1007/s10071-015-0872-2

Sato Y, Kano F, Hirata S (2018) Cutting-edge infrared thermography as a new tool to explore animal emotions. Jpn J Anim Psychol 68(1):1–15. https://doi.org/10.2502/janip.68.1.7

Seyfarth RM, Cheney DL (2013) Affiliation, empathy, and the origins of theory of mind. Proc Natl Acad Sci USA 110(Suppl 2):10349–10356. https://doi.org/10.1073/pnas.1301223110

Shenhav A, Mendes WB (2014) Aiming for the stomach and hitting the heart: dissociable triggers and sources for disgust reactions. Emotion 14(2):301–309. https://doi.org/10.1037/a0034644

Spikins P, Needham A, Tilley L, Hitchens G (2018) Calculated or caring? Neanderthal healthcare in social context. World Archaeol 50(3):384–403. https://doi.org/10.1080/00438243.2018.1433060

Tokuyama N, Emikey B, Bafike B et al (2012) Bonobos apparently search for a lost member injured by a snare. Primates 53(3):215–219. https://doi.org/10.1007/s10329-012-0298-2

Turner SE, Fedigan LM, Matthews HD, Nakamichi M (2014) Social consequences of disability in a nonhuman primate. J Hum Evol 68:47–57. https://doi.org/10.1016/j.jhevol.2014.01.002

van Baak MA (2008) Meal-induced activation of the sympathetic nervous system and its cardiovascular and thermogenic effects in man. Physiol Behav 94(2):178–186. https://doi.org/10.1016/j.physbeh.2007.12.020

Warren-Smith ÁB, Dunn WL (2006) Epimeletic behaviour toward a seriously injured juvenile bottlenose dolphin (*Tursiops sp.*) in Port Phillip, Victoria, Australia. Aquat Mamm 32(3):357–362. https://doi.org/10.1578/AM.32.3.2006.357

Webb CE, Romero T, Franks B, de Waal FBM (2017) Long-term consistency in chimpanzee consolation behaviour reflects empathetic personalities. Nat Commun 8(1):292. https://doi.org/10.1038/s41467-017-00360-7

Yamamoto S (2017) Primate empathy: three factors and their combinations for empathy-related phenomena. Wiley Interdiscip Rev Cogn Sci 8(3):e1431. https://doi.org/10.1002/wcs.1431

Zahn-Waxler C, Radke-Yarrow M, Wagner E, Chapman M (1992) Development of concern for others. Dev Psychol 28(1):126–136. https://doi.org/10.1037/0012-1649.28.1.126

2.9 Figures

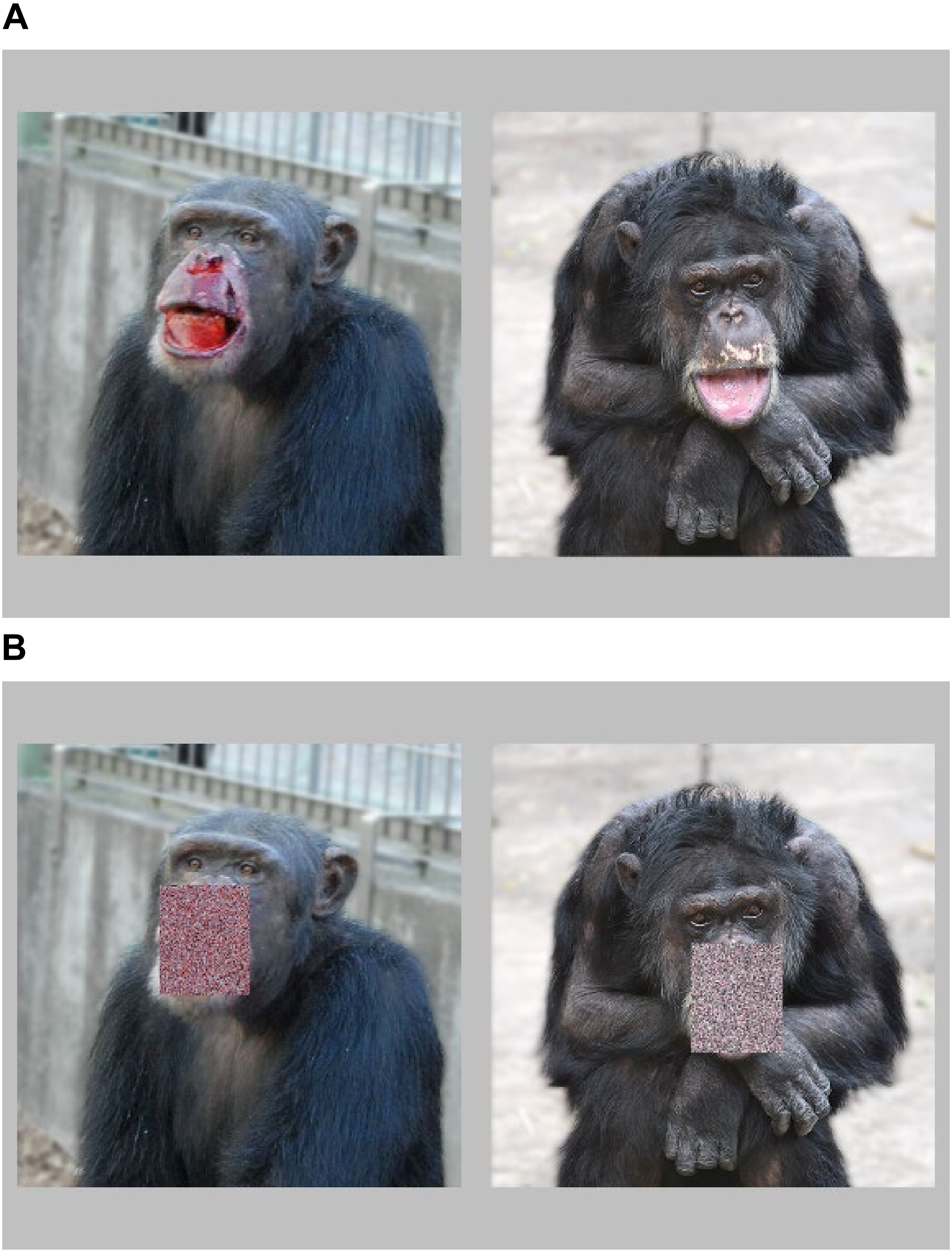

Fig. 2.1 **a** A stimulus used in the test condition. **b** The corresponding stimulus in the scramble condition.

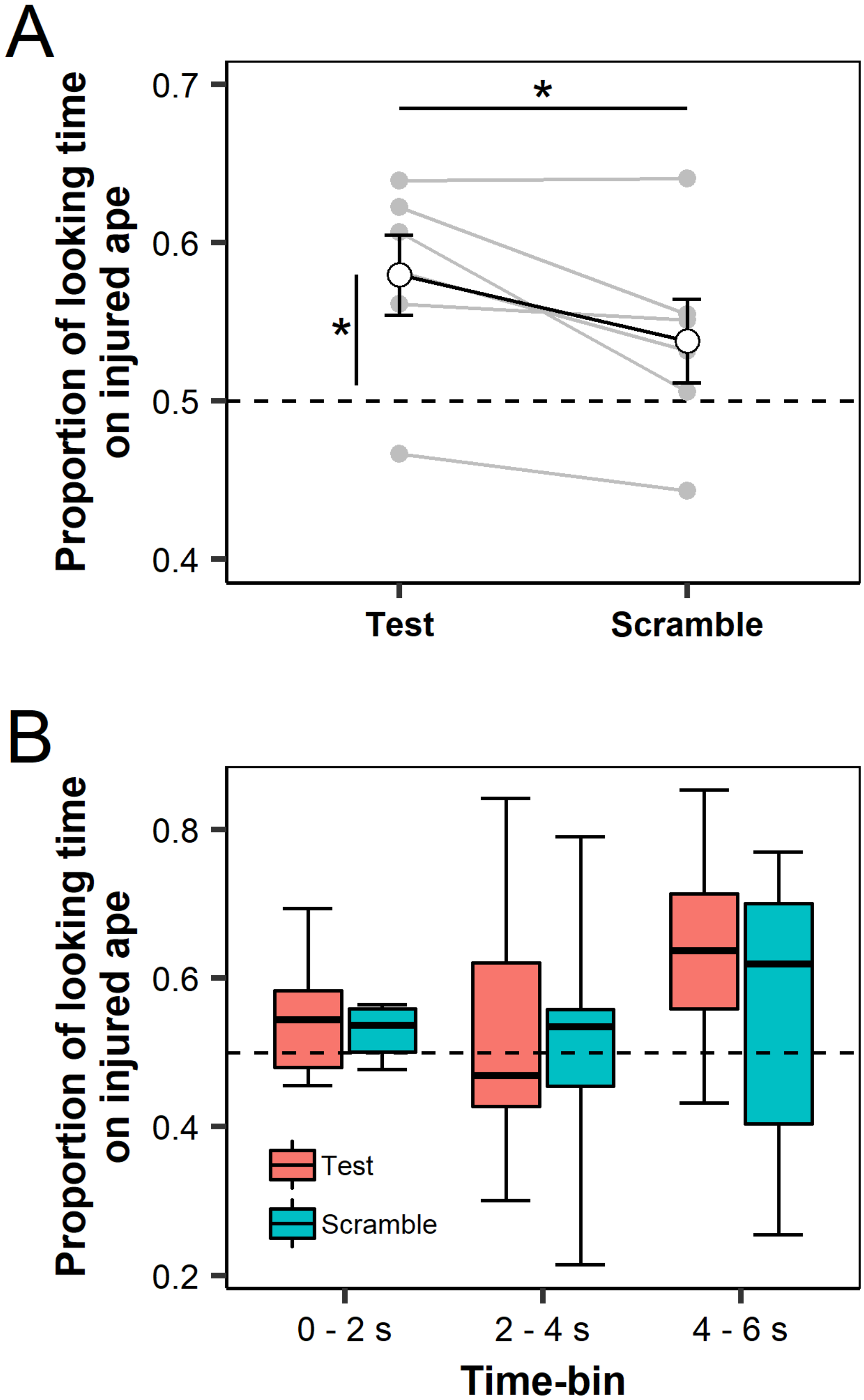

Fig. 2.2 **a** Proportion of time spent looking at injured chimpanzees (mean ± SEM, $n = 6$). Gray dots show each participant's data. The broken line indicates the chance level. **b** Proportion of looking time on injured chimpanzees in three time-bins ($n = 6$). The upper and lower ends of the box show the third- and first-quartiles, respectively. The thick line inside indicates the median. Whiskers indicate the maximum and minimum value. *: $p < 0.05$.

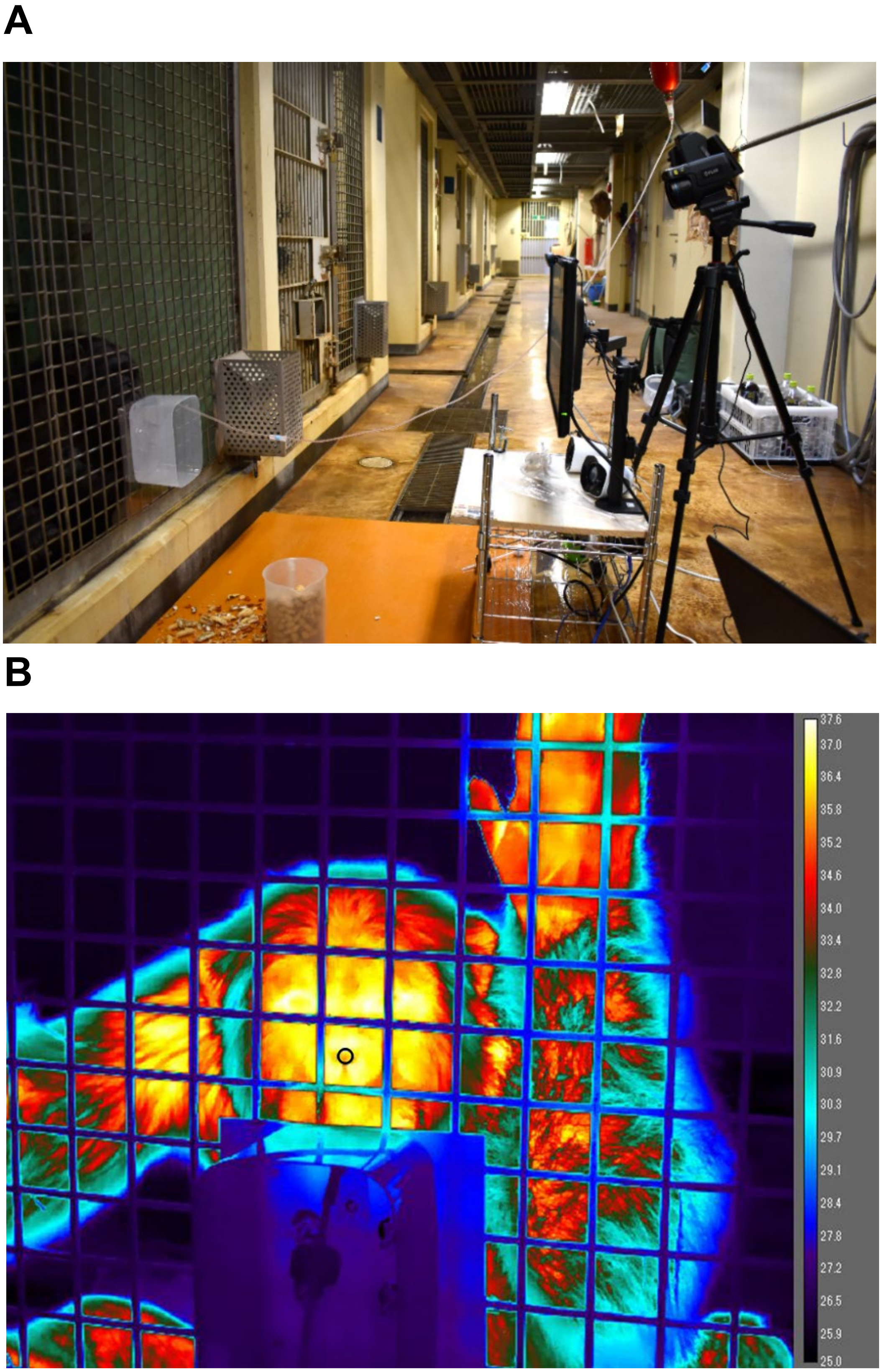

Fig. 2.3 Apparatus in preceding experiments (Supplementary Materials), Study 2 and Study 3. **a** Setup for the thermal imaging experiment. **b** An example of a thermal image. The AOI is

indicated as a black circle on the nasal tip. The color bar on the right hand indicates the temperature (°C).

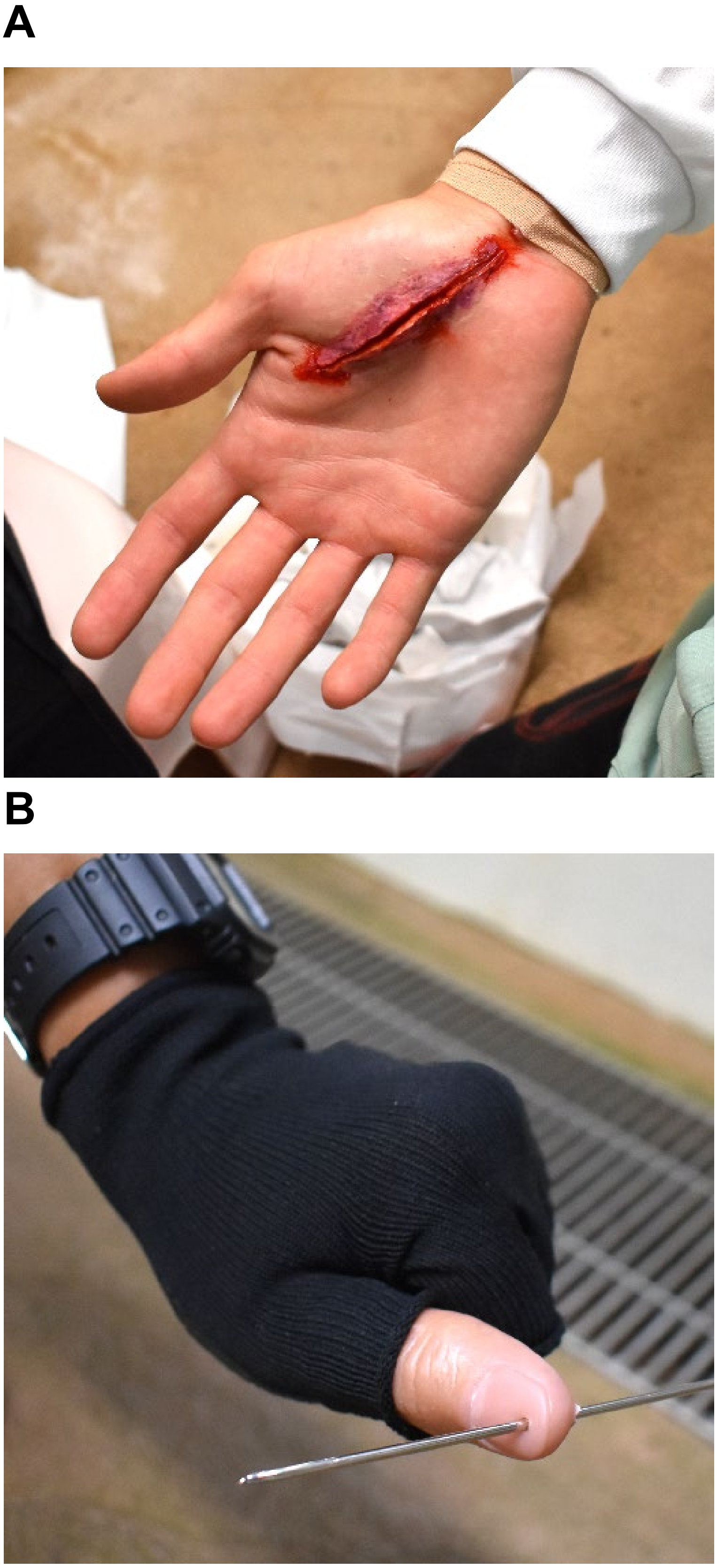

Fig. 2.4 Study 2 and Study 3 stimuli. **a** Injury make-up. Blood emerged from a tube hidden under the sleeve (the sleeve was slightly rolled up for visualization purposes in this photo). **b**

Fake thumb stabbed with a needle.

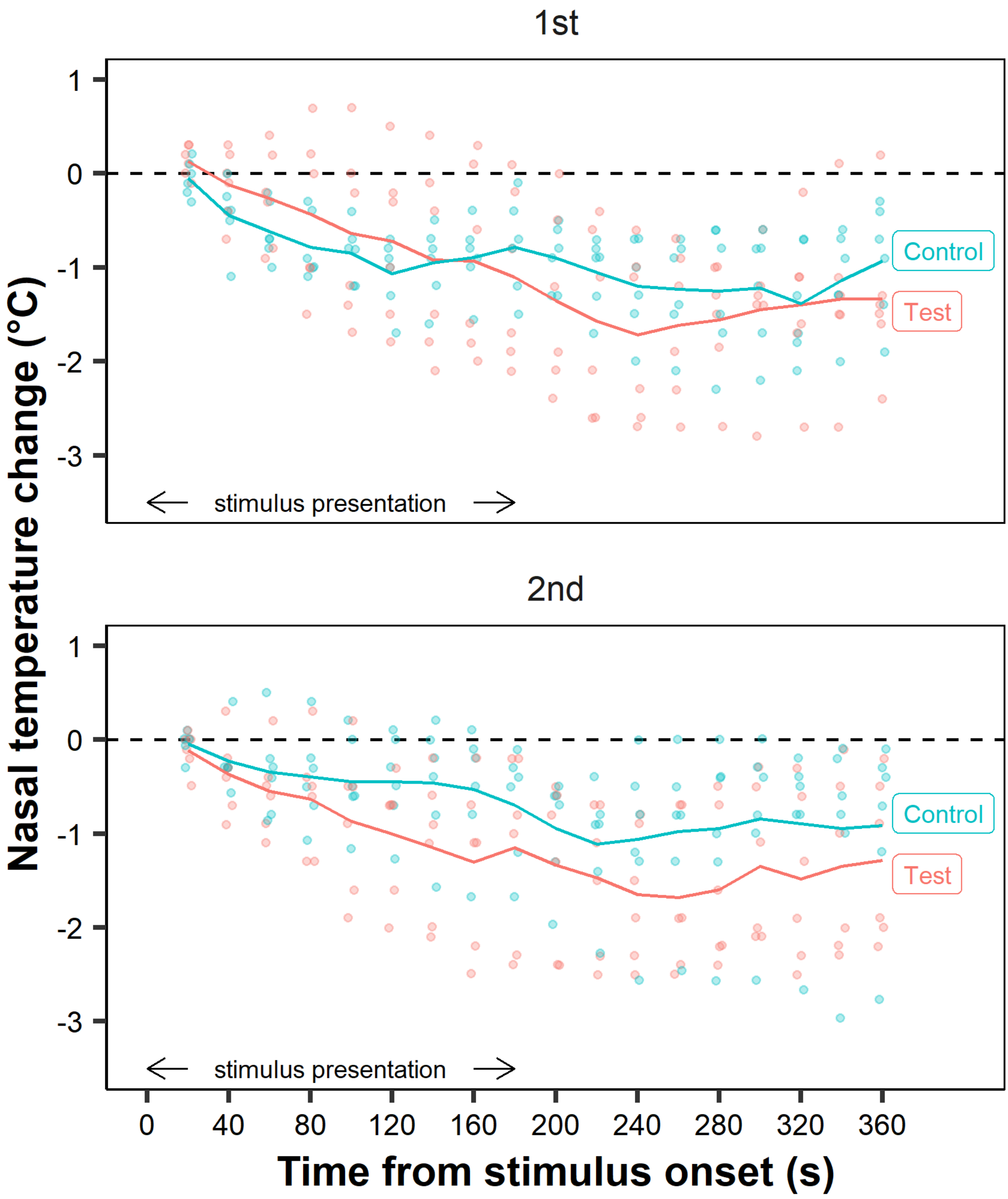

Fig. 2.5 Mean temperature change (°C) as a function of time from stimulus onset (s) in Study 2 (*n* = 6). Dots represent individual data.

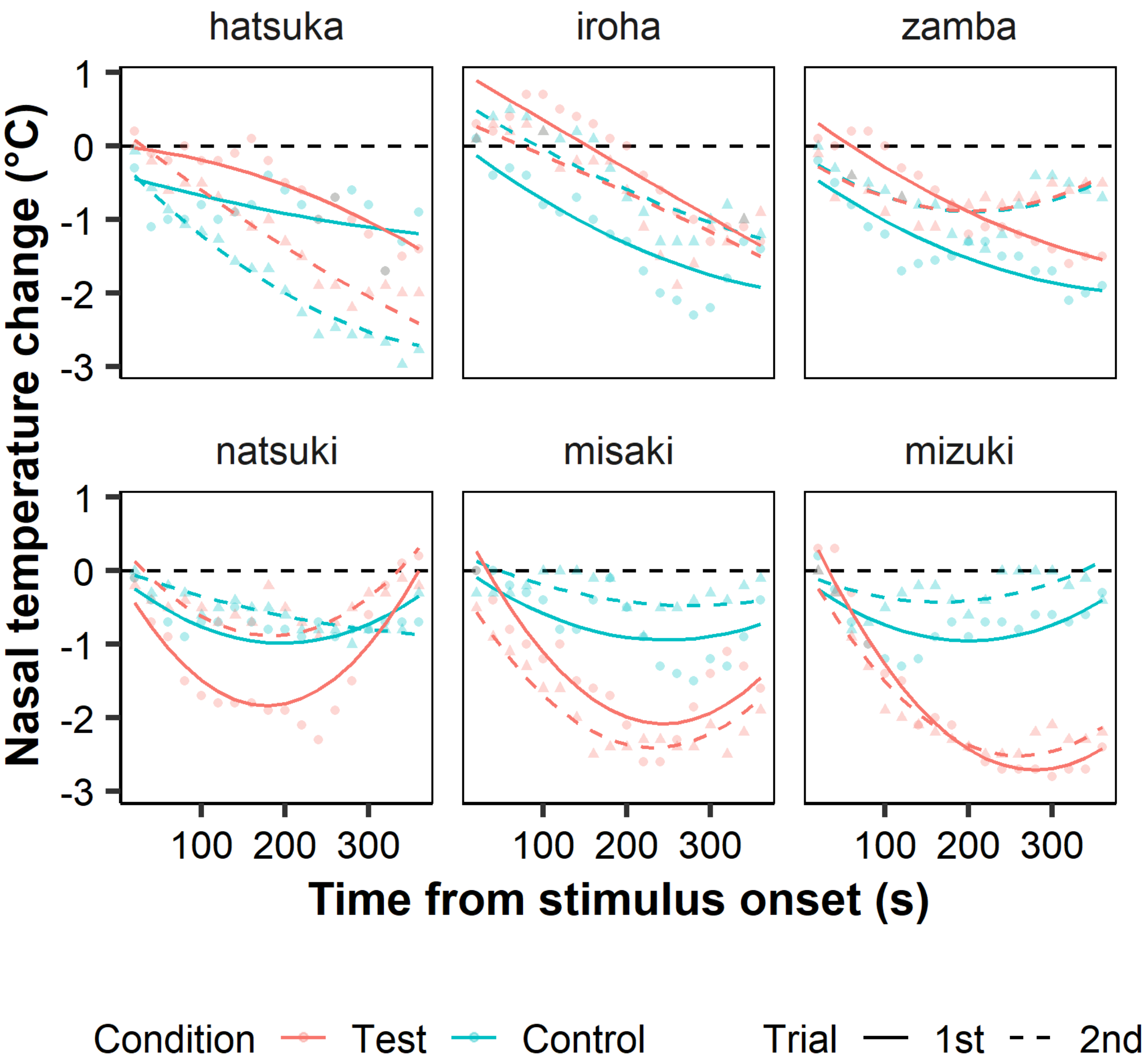

Fig. 2.6 Nasal temperature change (°C) of each chimpanzee participant in Study 2. Dots represent observed data and lines represent predicted values by the model. The top three panels show data of adolescent females (Hatsuka and Iroha: 9 years old) and an adult male (Zamba: 22 years old); and the bottom three panels show data of adult females (Natsuki: 12 years old; Misaki: 19 years old; Mizuki: 21 years old).

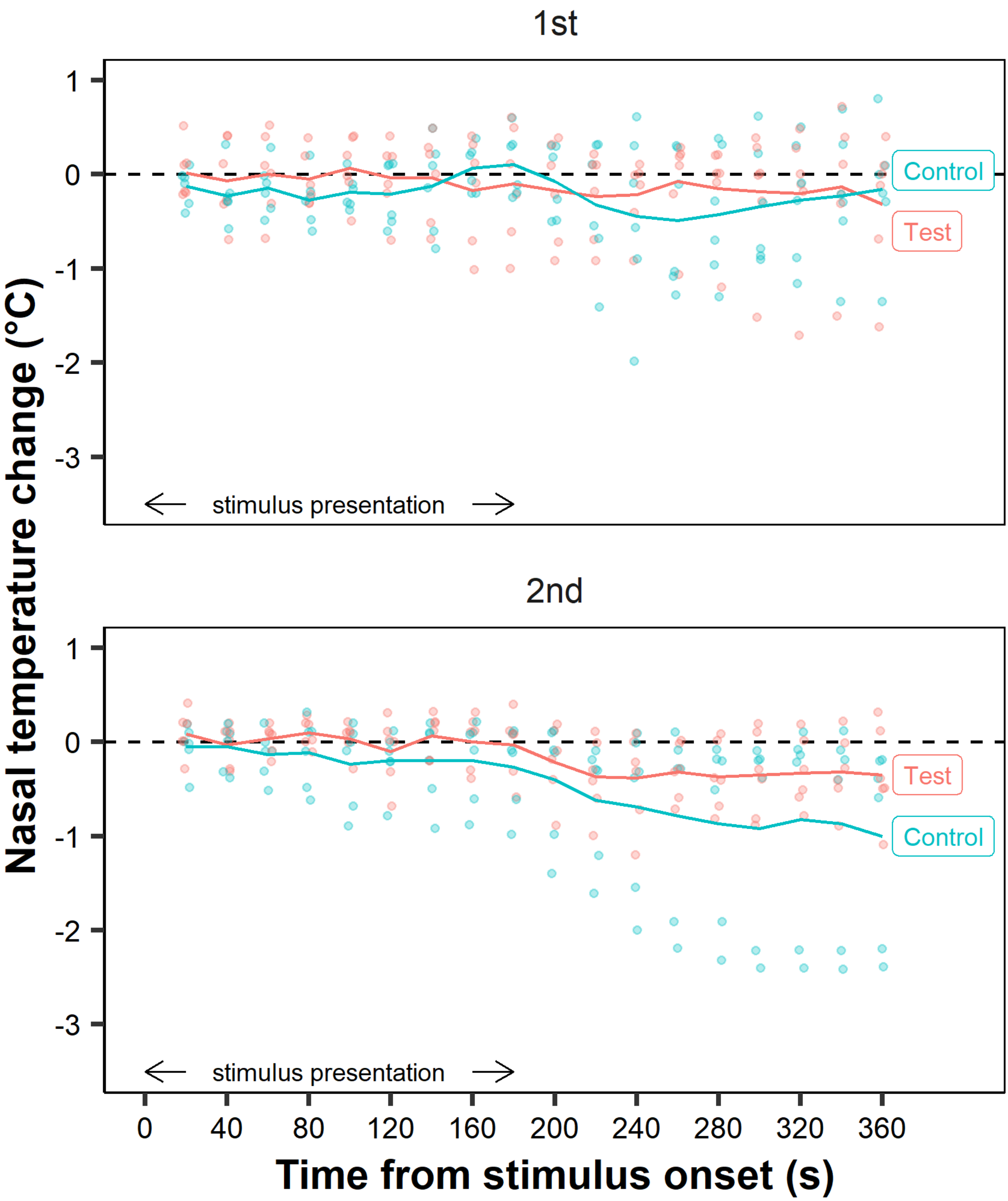

Fig. 2.7 Mean temperature change (°C) as a function of time from the stimulus onset (s) in Study 3 (*n* = 6). Dots represent individual data.

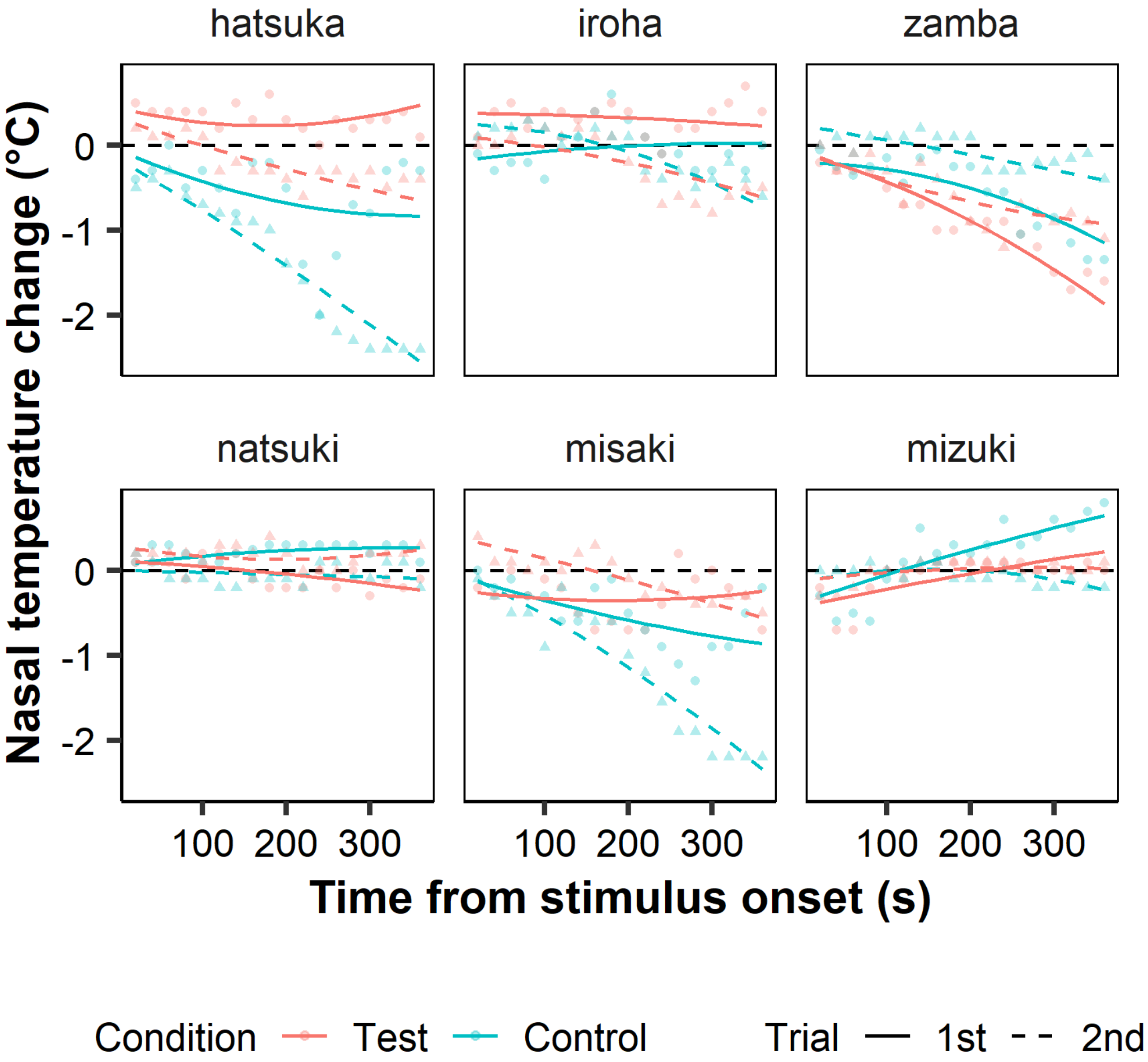

Fig. 2.8 Nasal temperature change (°C) of each chimpanzee participant in Study 3. Dots represent observed data, and lines represent predicted values by the model. The top three panels show data of adolescent females (Hatsuka and Iroha: 9 years old) and an adult male (Zamba: 22 years old); and the bottom three panels show data of adult females (Natsuki: 12 years old; Misaki: 19 years old; Mizuki: 21 years old).

2.10 Supplementary Materials

Table S2.1 Characteristics of the participants.

Name	Sex	Age	Rearing history	GAIN ID number*
Zamba	M	22	Mother	0543
Mizuki	F	21	Nursery/Peer	0559
Misaki	F	19	Mother	0593
Natsuki	F	12	Mother	0677
Hatsuka	F	9	Nursery/Peer	0704
Iroha	F	9	Mother	0708

Note. In the second column, “M” refers to males and “F” refers to females. Rearing history is shown in the last column, with “Mother” means reared by their mother and “Nursery/Peer” means reared by human caretakers and conspecific peers. *: Great Ape Information Network (https://shigen.nig.ac.jp/gain/index.jsp)

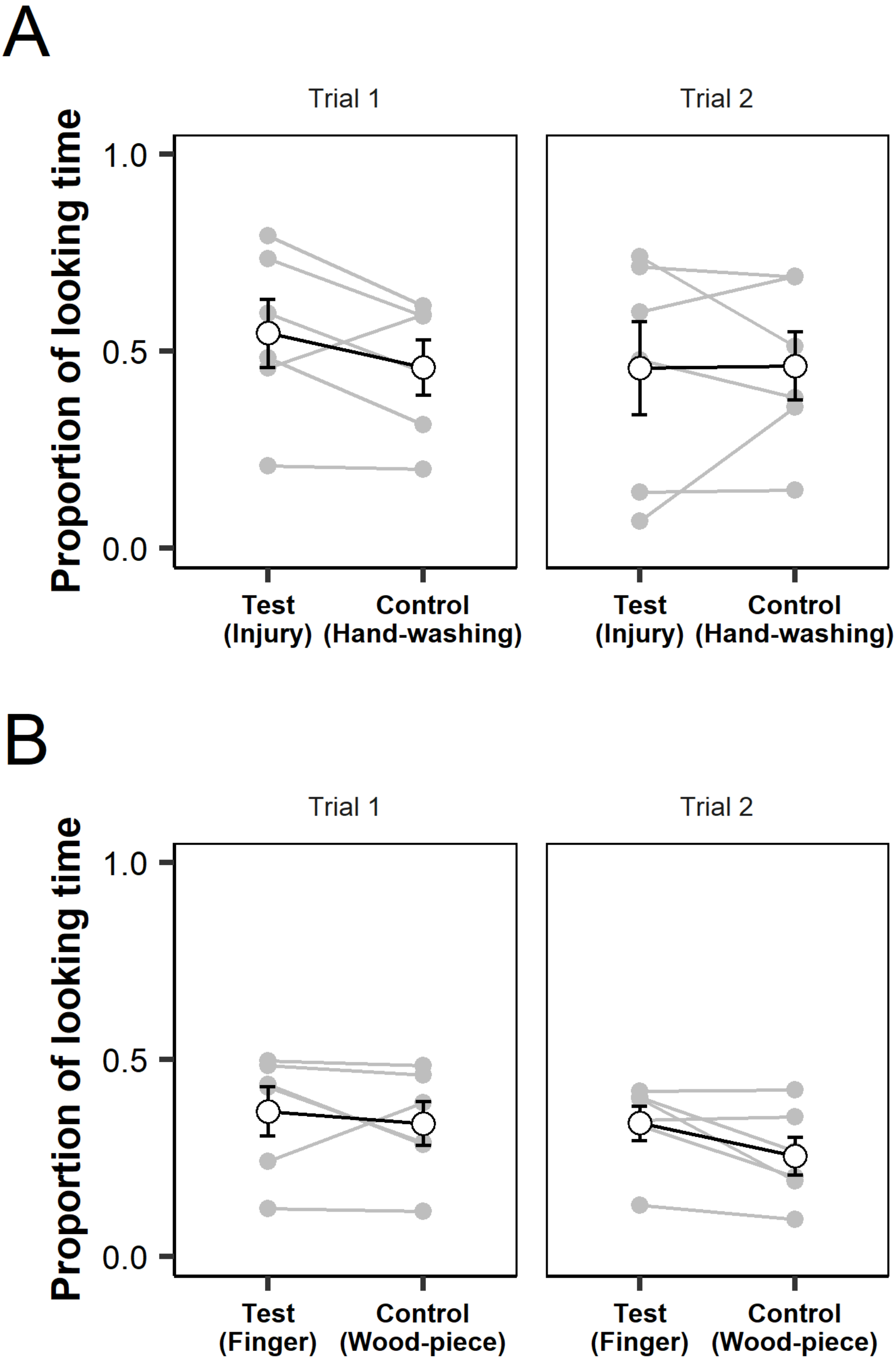

Fig. S2.1 The proportion of time spent looking at the stimulus (A) in Study 2 and (B) in Study 3 (mean ± SEM, *n* = 6). Gray dots represent individual data.

Table S2.2 Mean ± SD duration of behavioral items (s; *n* = 6).

	Condition	Trial	(1) Looking at stimulus	(2) Leaving juice dispenser	(3) Not clearly seen
Study 2	Test	1	84.12 ± 41.07	6.04 ± 12.55	21.12 ± 22.90
		2	76.22 ± 50.34	1.39 ± 3.40	14.71 ± 16.86
	Control	1	54.13 ± 16.80	4.80 ± 11.76	50.35 ± 33.83
		2	67.81 ± 43.13	-	37.64 ± 47.46
Study 3	Test	1	56.59 ± 26.69	-	24.56 ± 30.45
		2	56.85 ± 18.04	-	10.84 ± 10.28
	Control	1	58.83 ± 24.33	-	5.82 ± 5.25
		2	44.09 ± 20.50	-	6.76 ± 6.22

Table S2.3 The results of LMM in Study 2.

Variables	*β*	*SE*	*df*	*t*	*p*
(Intercept)	–0.01	0.11	9.80	–0.13	0.899
Condition	0.33	0.12	9.40	2.81	0.019
Trial	0.22	0.16	14.60	1.40	0.183
Time	–0.14	0.02	19.70	–8.63	0.000
Time^2	0.00	0.00	13.90	4.72	0.000
Condition × trial	–0.56	0.30	12.70	–1.87	0.085
Condition × time	–0.12	0.07	6.40	–1.78	0.123
Condition × time^2	0.00	0.00	6.30	1.45	0.194
Trial × time	0.02	0.05	9.70	0.36	0.730
Trial × time^2	0.00	0.00	10.10	–0.48	0.640
Condition × trial × time	0.01	0.04	358.70	0.30	0.767
Condition × trial × time^2	0.00	0.00	358.70	0.31	0.758

Note. Each variable was coded as follows; condition: control = 0, test = 1; trial: 1st = −0.5, 2nd = 0.5; time: exact time (s) divided by 20 (e.g., 20 s = 1). Significant variables ($p <$ 0.05) are highlighted in bold.

Table S2.4 The results of LMM in Study 3.

Variables	*β*	*SE*	*df*	*t*	*p*
(Intercept)	–0.05	0.07	10.80	–0.75	0.467
Condition	0.14	0.11	10.50	1.32	0.214
Trial	0.16	0.14	18.80	1.20	0.244
Time	–0.02	0.03	7.50	–0.80	0.451
Time^2	0.00	0.00	14.50	–1.11	0.283
Condition × trial	–0.04	0.22	14.50	–0.20	0.848
Condition × time	–0.01	0.02	25.10	–0.28	0.783
Condition × time^2	0.00	0.00	39.00	1.19	0.242
Trial × time	–0.03	0.02	373.30	–1.27	0.205
Trial × time^2	0.00	0.00	42.10	–0.75	0.459
Condition × trial × time	0.01	0.03	373.30	0.31	0.758
Condition × trial × time^2	0.00	0.00	373.30	0.76	0.448

Note. Each variable was coded as follows; condition: control = 0, test = 1; trial: 1st = −0.5, 2nd = 0.5; time: exact time (s) divided by 20 (e.g., 20 s = 1).

Preceding Experiments

We used infrared thermal imaging to examine chimpanzees' physiological responses to still images of injured conspecifics (Experiment S1) and video clips of conspecifics being injected with hypodermic needles (Experiment S2).

Methods

Participants

The same chimpanzees as in Study 1 participated in the pilot experiments.

Apparatus

The apparatus used was identical to those used in Study 2 and 3, except that we used a 23-inch LCD monitor to present stimuli and a web camera instead of a video camera to record participants' looking behavior.

Stimuli and procedures

For Experiment S1, we prepared 15 images of injured chimpanzees as test stimuli and 15 images of uninjured chimpanzees as control stimuli (1280 × 720 pixels). We selected those images and did not use the same images used in Study 1. In the control images, the depicted chimpanzees were different from those depicted in the test condition, and sex was matched between conditions. The depicted chimpanzees were eating food, based on previous experience that images of foraging conspecifics capture chimpanzee participants' attention without making them aroused. As in Study 1, the depicted body parts and postures were matched as closely as possible between conditions. All depicted chimpanzees were unfamiliar to participants. Each trial contained two blocks and lasted for 180 s. Each block lasted for 90

s. Each image was presented for 6 s (6 s × 15 images) once in each block (and twice in each trial).

For Experiment S2, we prepared five 10-s movie clips depicting an unfamiliar chimpanzee (under anesthesia) being injected with a needle as test stimuli (1280 × 720 pixels). The depicted chimpanzees were unfamiliar to participants. As control stimuli, we prepared five 10-s movie clips of a familiar chimpanzee (one of the participants) being gently touched by a keeper. All movies had no sound. Each trial had three blocks and lasted for 150 s. Each block lasted for 50 s. Each movie lasted for 10 s (10 s × 5 clips).

The images and movies were processed using Adobe Photoshop CC 2017 and Adobe Premiere Pro CC 2017. In both experiments, we conducted two sessions for each condition. Each session involved one trial each day (total four days/sessions per experiment). The order of condition was counterbalanced across participants. We analyzed video data of the web-camera following the same procedures as in Study 2 and 3.

We conducted the experiments between 11 a.m. to 2. p.m., in April and May 2018. The room temperature and humidity at the time of testing (mean ± SD) were 19.7 ± 1.3 °C and 62.3 ± 6.6% in Experiment S1; 20.4 ± 0.7 °C and 54.2 ± 10.9% in Experiment S2, respectively.

Results and Discussion

Overall, chimpanzees did not show different patterns of change in nasal temperature across conditions. Although we observed changes in nasal temperature in several trials, the observed patterns of changes were largely irrelevant of the stimulus presentation (Fig. S2 and Fig. S3 for Experiment S1 and Experiment S2, respectively). There are several possible reasons for such changes. First, such changes may have been caused by confounding factors, such as relatively low baseline skin temperatures. Such low baseline skin temperatures were likely to have been caused by relatively low air temperature in that season, when we conducted the pilot experiments (Fig. S4). Second, these changes may have been caused by participants' arousal upon hearing fights in other chimpanzee groups at the sanctuary. Nonetheless, if there were a strong effect of the presented stimuli, we would have observed such an effect. One potential reason for the lack of such an effect is that the presented stimuli may not have been sufficiently realistic and/or powerful to elicit arousal. Therefore, in Study 2 and 3, we made the following improvements: (1) we conducted the experiments after winter, and tested whether the participants' nasal temperature recovered relatively rapidly (in a few minutes) when they came into the indoor sleeping room; (2) we extended the post-stimulus period to 3 min; (3) we used real-life theatrical demonstrations.

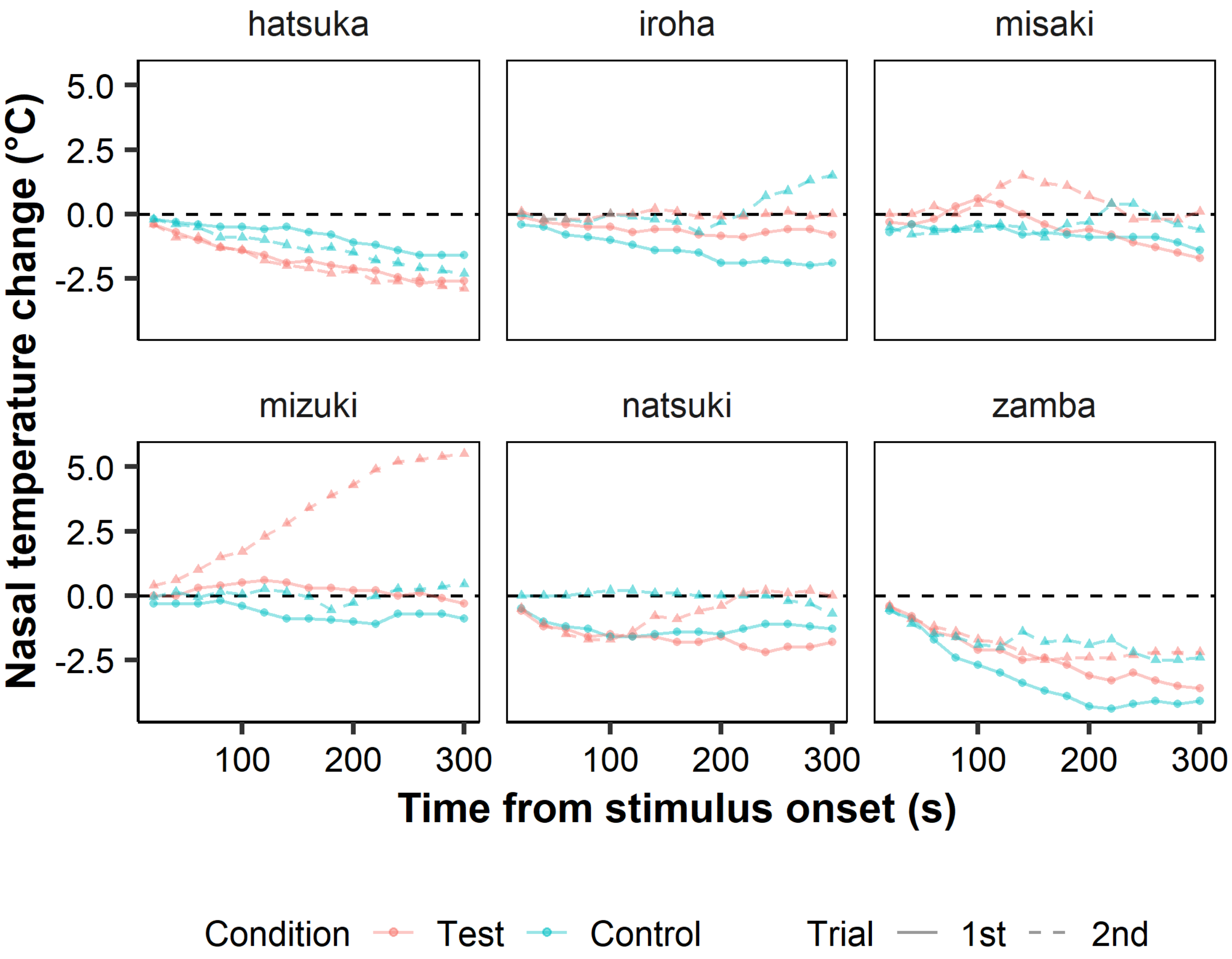

Fig. S2.2 Nasal temperature change (°C) as a function of time from stimulus onset (s) in Experiment S1 (*n* = 6). Stimulus presentation lasted for 180 s. Panels show each participant's data.

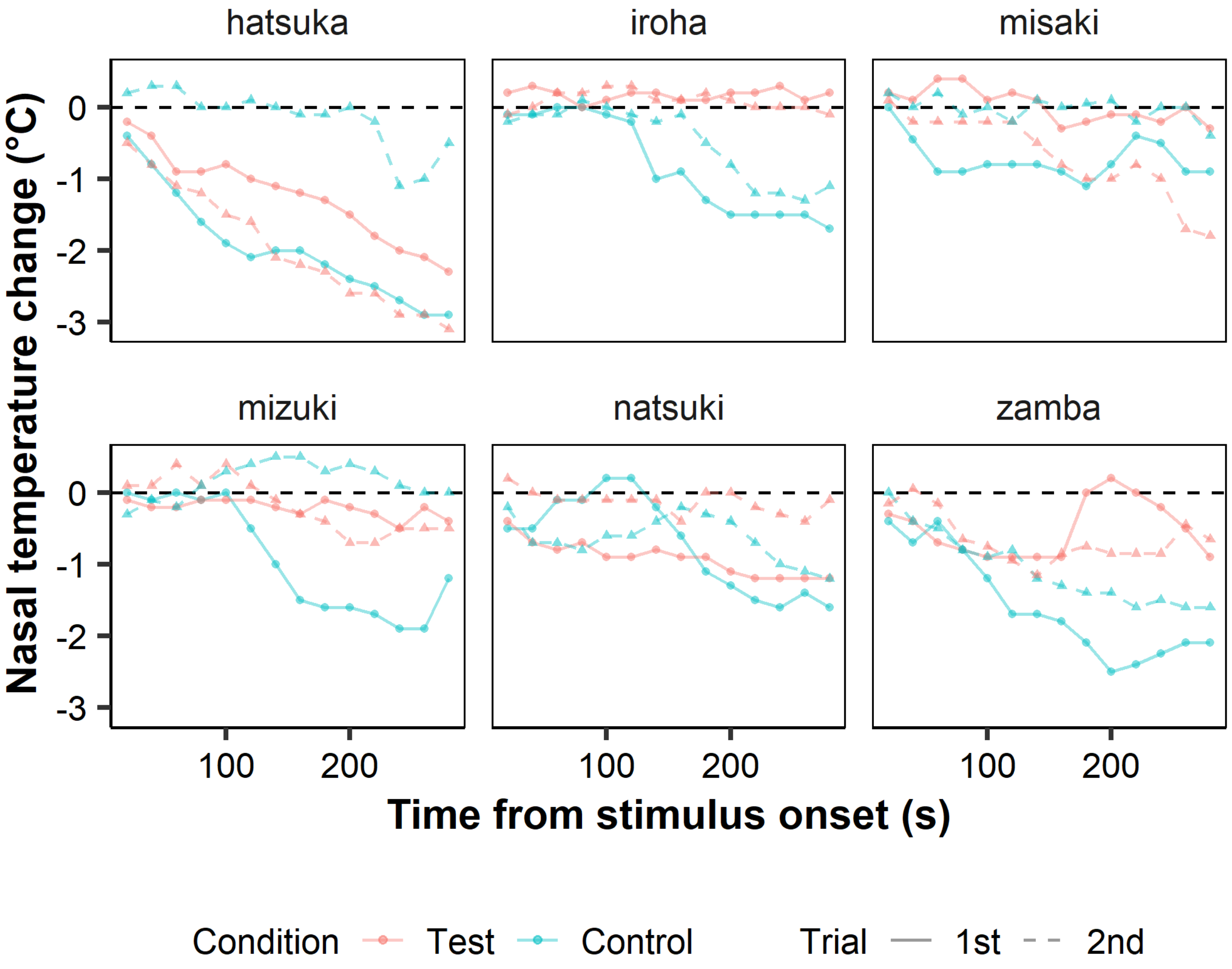

Fig. S2.3 Nasal temperature change (℃) as a function of time from stimulus onset (s) in Experiment S2 (*n* = 6). The demonstration/stimuli lasted for 150 s. Panels show each participant's data.

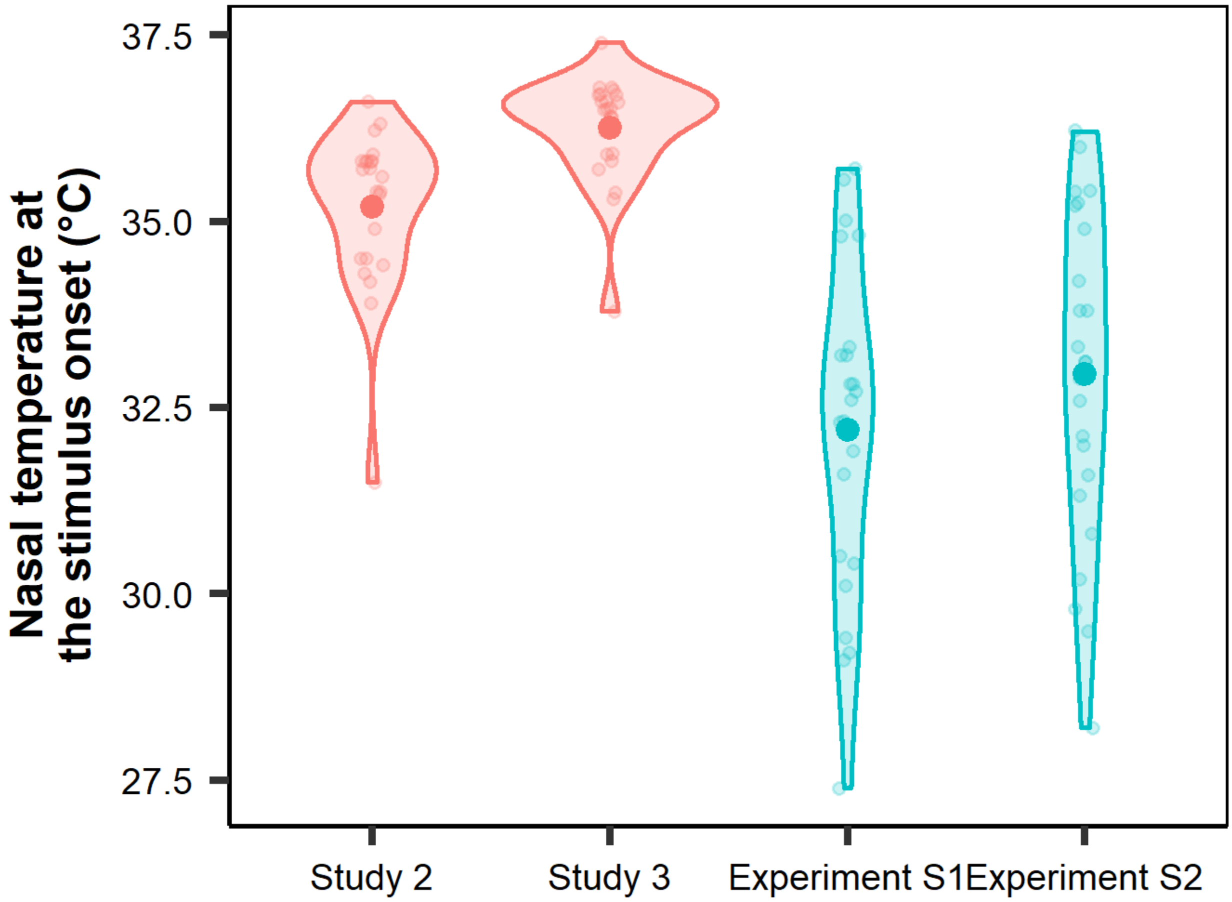

Fig. S2.4 Violin plots showing the baseline temperature in each experiment. Smaller dots show individual data (6 participant × 2 conditions × 2 trials = 24 data for each experiment), and larger ones show the mean.

Chapter 3

Great Apes' Understanding of Biomechanics: Eye-Tracking Experiments Using Three-Dimensional Computer-Generated Animations

3.1 Abstract

Visual processing of the body movements of other animals is important for adaptive animal behaviors. It is widely known that animals can distinguish articulated animal movements even when they are just represented by points of light such that only information about biological motion is retained. However, the extent to which nonhuman great apes comprehend the underlying structural and physiological constraints affecting each moving body part, i.e., biomechanics, is still unclear. To address this, we examined the understanding of biomechanics in bonobos (*Pan paniscus*) and chimpanzees (*Pan troglodytes*), following a previous study with humans (*Homo sapiens*). Apes underwent eye-tracking while viewing three-dimensional computer-generated (CG) animations of biomechanically possible or impossible elbow movements performed by a human, robot, or nonhuman ape. Overall, apes did not differentiate their gaze between possible and impossible movements of elbows. However, some apes looked at elbows for longer when viewing impossible vs. possible robot movements, which indicates that they may have had knowledge of biomechanics and that this knowledge could be extended to a novel agent. These mixed results make it difficult to draw a firm conclusion regarding the extent to which apes understand biomechanics. We discuss some methodological features that may be responsible for the results, as well as implications for future nonhuman animal studies involving the presentation of CG animations or measurement of gaze behaviors.

Keywords: Biomechanics, Body movement, Bonobo, Chimpanzee, Computer-generated animation

3.2 Introduction

Visual processing of the movements of other animals is important for adaptive behaviors (e.g., Johansson 1973; Nakayasu and Watanabe 2014; Simion et al. 2008). To assess the perception of biological motion (BM), the kinematics of body movements are often presented as point-light displays (PLDs) (Johansson 1973). Animals can differentiate PLDs representing BM from other control PLDs to some extent (e.g., Blake 1993; De Agrò et al. 2021; Nakayasu and Watanabe 2014; Regolin et al. 2000). Such abilities are observed even in newborn animals such as newly hatched chicks (*Gallus gallus*) (e.g., Regolin et al. 2000; Vallortigara and Regolin 2006; Vallortigara et al. 2005) and human newborns (*Homo sapiens*) (Bardi et al. 2011, 2014; Bidet-Ildei et al. 2014; Simion et al. 2008). Vallortigara et al. (2005) found that chicks spontaneously approached PLDs displaying the BM of conspecifics (hen), hetero-specifics (cat), and vertebrate-like scramble motion, which suggests that chicks have some innate detection mechanism for vertebrate motion. Simion et al. (2008) also found that newborn human babies preferentially looked at hetero-specific (hen) BM, which suggests that such innate reactivity may be shared over a wide range of animal taxa.

Furthermore, several laboratory experiments have suggested that nonhuman animals can detect abnormal biomechanical movement to some extent. Computer-generated (CG) animation is particularly useful for displaying impossible movements that cannot be performed by living animals (Campbell et al. 2009; Chouinard-Thuly et al. 2017; Watanabe and Troje 2006). Watanabe and Troje (2006) conducted a study using CG animations of pigeons, and found that pigeons appeared to learn to discriminate between normal conspecifics and conspecifics with abnormal movements (hopping without head-bobbing) by attending particularly to head movements. Similarly, Nakayasu et al. (2017), who used CG fish animations, observed reduced social approach behaviors in medaka fish (*Oryzias latipes*) when shown conspecifics with atypical locomotion compared with normal conspecifics. These studies used CG animations to successfully uncover new information regarding the understanding of body movements in animals.

However, this line of research has not yet included our closest evolutionary relatives, nonhuman great apes (hereafter referred to as “apes”). Regarding the understanding of body structure, a chimpanzee (*Pan troglodytes*) differentiated a PLD representing BM from a randomized version in a visual search task, although such differentiation was not limited to BM (Tomonaga 2001). Furthermore, Gao and Tomonaga (2020a) examined the effect of image inversion on recognition performance (i.e., the inversion effect). Their findings indicated that chimpanzees used configural processing for images of a normal body, but not for those of a body with scrambled body part locations, which indicates that they may have knowledge regarding the position of each body part [for a similar finding with capuchin monkeys, see Matsuno and Fujita (2018)]. However, the researchers did not directly examine the discrimination of normal from abnormal bodies (Gao and Tomonaga 2020a), and they used static images without motion cues. Thus, little is known regarding how apes understand the unique structural and physiological constraints underlying dynamic body movements, namely, biomechanical restraints. Such information is critical for distinguishing body movement processing between nonhuman animals and humans.

Humans appear to be capable of distinguishing biomechanically possible and impossible movements of particular body parts from early ontogeny (Geangu et al. 2015; Longhi et al. 2015; Morita et al. 2010, 2012; Reid et al. 2005, 2008; Senna et al. 2017). To investigate the underlying developmental process, Morita et al. (2012) carried out a cross-sectional study in which human adults and infants were presented with CG animations of biomechanically possible or impossible elbow movements (i.e., bending upward or downward, respectively), performed by either a human or robot. They found that adults and

12-month-old infants, but not 9-month-old infants, looked at the impossible elbow movements for longer than the possible movements in both the human and robot animations. This indicates that both adults and infants have knowledge regarding the biomechanics of human movement, and that they extend this to the movement of nonhuman agents with a similar structure [for similar studies, see: Komori et al. (2006) and Morita et al. (2010)]. Additionally, the adults exhibited pupil dilation, which is a physiological indicator of affective arousal, when viewing impossible vs. possible human movements. This could be associated with emotional responses such as surprise, fear, or disgust, as self-reported by another participant group (Morita et al. 2010). In view of this, and in conjunction with the previous finding that observing the movements of others activates sensorimotor representation for the observed movements [e.g., Iacoboni et al. 1999; cf. a recent review by Riečanský and Lamm (2019) on pain and empathy], Morita et al. (2012) concluded that adults identify biomechanical implausibility on the basis of such mirror-like mechanisms as well as knowledge regarding human movements acquired via experience, whereas 12-month-old infants do so primarily on the basis of the latter. This interpretation of the pupillometry data was in accord with other experiments suggesting that the mirroring of somatic components of observed actions might play a role in distinguishing biomechanically possible and impossible movements (Avenanti et al. 2007; Costantini et al. 2005; Romani et al. 2005). Here, based on that study of Morita et al. (2012), we carried out a series of eye-tracking experiments to examine how apes understand biomechanics. Specifically, like in Morita et al. (2012), ape participants were presented with CG animations of a human and a robot displaying biomechanically possible or impossible arm movements, while participants underwent eye tracking (experiments 1 and 3). We also presented the participants with ape animations displaying similar movements (experiments 2 and 3). We assumed that if the apes looked at the elbow regions for longer when viewing impossible vs. possible movements, then this could indicate that they understood how the elbow should move. That is, the increased attention could reflect a novelty or a violation of predictions, as is usually assumed in similar developmental psychology studies (e.g., Longhi et al. 2015; Morita et al. 2012; Reid et al. 2005).

Pupillometry is known to be challenging in nonhuman animals mainly because of difficulties with head fixation [Massen et al. 2019; but see Hepach et al. (2021) for an attempt at using pupillometry to examine arousal in chimpanzees]. Our pupillometry results may have been affected by the visual properties of the stimuli (see *General Discussion*). Thus, unlike Morita et al. (2012), we mainly reported ape gaze behaviors. However, we included a brief summary of our pupillometry results to encourage future implementation of this technique, which may facilitate the examination of ape affective responses associated with autonomic nervous system activity [Kret et al. 2020; for a review on human and monkey studies see Peinkhofer et al. (2019)]. To this end, we provided detailed data visualization in Supplementary file 1 (Fig. S3.10−S3.18).

3.3 Experiment 1

First, we presented apes with human and robot animations that were slightly modified from those used in Morita et al. (2012). The participant apes lived with conspecific groupmates and had observed human movements via daily interactions with caretakers, researchers, or visitors. If apes have some understanding of human biomechanics, they should look at human elbows for longer when viewing impossible vs. possible movements. Furthermore, if apes have some understanding of biomechanics and, like humans, can infer the biomechanics-like properties of a novel robot agent, then they should also look at the elbows of the robot animation for longer when viewing impossible vs. possible movements. Note that it was unlikely that the apes would make inferences based on the direct experience of seeing similar robotic agents because the apes had rarely seen a robot or watched a three-dimensional CG animation of a robot.

3.3.1 Methods

Participants

Sixteen apes participated in experiment 1: six bonobos (*Pan paniscus*; four females, two males; range 15−46 years old) and six chimpanzees (five females, one male; range 10−23 years old) at Kumamoto Sanctuary, Wildlife Research Center, Kyoto University (KS); and four chimpanzees (one female, three males; range 6−42 years old, including estimated age) at Kyoto City Zoo (KCZ) (Supplementary file 1, Table S3.1). The experiments depended on voluntary participation, and the apes were not subjected to deprivation of food or water.

Apparatuses

The apes underwent eye tracking using an infrared eye tracker [300 Hz, Tobii TX300, Tobii Technology (KS); 60 Hz, Tobii X120, Tobii Technology (KCZ)]. Movie stimuli were presented on a 23-in (approx. 58 cm) liquid crystal display monitor with a viewing distance of approximately 70 cm, and sounds were played from speakers. The eye tracker and speaker were mounted under the monitor. The apes viewed the monitor through a transparent panel [1-cm-thick polycarbonate panel (KS); 2-cm-thick acrylic panel (KCZ)]. On each day, anti-fog spray was applied to the panel so that neither the view nor the eye tracker recordings were obscured by condensation created by the breath of the participants. Bonobos and chimpanzees at KS participated in the experiments alone or in a dyad in an indoor enclosure (2.8 m × 5.4 m × 3.5 m; Fig. 3.1a), or in an experimental booth (3 m × 3 m × 2 m), respectively. Chimpanzees at KCZ participated in an indoor enclosure (~ 18.47 m^2 that included separate surrounding rooms for human experimenters; Fig. 3.1b) that was accessible to all their groupmates during testing. The apes viewed the monitor while sipping juice from a nozzle attached to the panel. Juice was delivered from a custom-made juice dispenser hanging on the wall or from a bottle through a straw. In KS, a human experimenter softly held the head of some of the chimpanzees during the session, and the session was conducted without juice for one individual. The juice served to reduce the head motion of participants during the eye tracker recordings. We did not adjust the amount of juice delivered according to a participant's gaze, so the juice did not serve as a reinforcer like in previous relevant eye-tracking experiments.

Stimuli and Procedure

We adjusted the size and duration of the original stimulus materials used in Morita et al. (2012). Specifically, we made 6-s animations (1920 × 1080 pixels) in which either a human or a robot figure repeatedly (1) held both arms forward, (2) bent both elbows, and (3) stretched both arms out every 1.5 s (Fig. 3.2a, b). These animations are shown in Supplementary files 2−5.

Before the first session for each participant, we performed two-point automated calibration in which we presented participants with a small image at two corners of the screen one by one. We also performed the calibration whenever necessary. To assess the calibration accuracy, at the start of each session, nine images were presented on the screen and an experimenter manually compared the position of the recorded gaze with one of these images. The experimenter occasionally showed a real object by hand in front of the image on the screen to attract the participants' attention to the position of the image. We sought to maintain recording accuracy such that recording errors were typically within 1° (Kano and Tomonaga 2009; Kano et al. 2011; Hirata et al. 2010). Nevertheless, the errors may have been larger at KCZ because the eye tracker angle may have occasionally been different from that during calibration, and the calibration accuracy could not be checked clearly due to participants' restlessness (but in a few cases).

Each trial comprised a 6-s animation and a session included four trials, such that the participants viewed all four animations in a session (i.e., human and robot animations displaying possible and impossible movements). Each participant underwent two sessions on separate days; data were missing for the second session for one participant at KCZ. The order of the four trials within a session was counterbalanced across participants using Tobii Studio software, which generated the same number of order patterns as there were trials using a Latin Square, and assign one pattern to each participant. The animations were presented to the chimpanzees with accompanying sounds that were timed to accompany the arm movements to attract attention [like stimuli for infants in Morita et al. (2012)]. A 2-s attention catcher (several moving illustrations with sounds) preceded each animation. Sounds were not played to the bonobos because they are known to become nervous upon hearing unfamiliar sounds. The calibration, gaze and pupil diameter recordings, and stimulus presentation were performed using Tobii Studio software v.3.4.8 (or v.3.2.3 for bonobos).

Analyses

Following Morita et al. (2012), we analyzed the data from the first trial of each animation to reduce the potential influence of stimuli habituation. Indeed, we observed reduced gaze time towards the screen from the first to the second presentation, irrespective of the models or movements (see Supplementary file 1, Figs. S3.1−S3.3 for the distributions of screen viewing time). The animations in this study were monotonous, as they depicted a repetition of the same arm movements. Thus, the apes may have rapidly habituated to the animations. Some apes also appeared more restless during the second vs. the first presentation. When participants did not view the stimuli for ≥ 1.5 s in the first trial, but did so in the second trial, we used the data from the second trial because it seemed inappropriate to include data from trials in which the apes were not attentive to the stimuli. We decided upon this criterion after considering that each action sequence took 1.5 s to complete (i.e., it took approximately 1.5 s for the position to change from holding both arms forward, bending both elbows, and stretching both arms out again), and that stricter criteria would reduce the sample size to a large extent (see Supplementary file 1, Figs. S3.1−S3.3). We also analyzed the first and second trials separately while excluding trials with a screen viewing time of < 1.5 s, and confirmed that the overall results, reported here, matched the analysis of the first trial only (summarized in Supplementary file 1, Table S3.2, Figs. S3.4−S3.6).

Figure 3.2 shows each area of interest. For each trial, we calculated the proportion of time spent looking at the elbows (i.e., elbow gaze time divided by the total time spent viewing the screen) and compared it between possible and impossible movements separately for the human and robot animations. We conducted statistical analyses using R software v.4.0.2 (R Core Team 2020). We a Wilcoxon signed-rank test (two-tailed, $\alpha = 0.05$) with the exactRankTests v.0.8.31 R package (Hothorn and Hornik 2019). We used a non-parametric test for the proportion of gaze time because this variable had a heavily skewed distribution. We also calculated Cliff's d as the effect size statistic (Cliff 1993) using the orddom v.3.1 R package (Rogmann 2013). Cliff's d ranges from −1 to 1, increases in value as more participants exhibit a difference in an expected direction (i.e., a higher proportion of elbow gaze time when viewing impossible vs. possible movements) and vice versa, and takes a value of 0 when there is no such difference. Asymmetric confidence intervals (CIs) were calculated based on the effect size and a variance estimate [Feng and Cliff (1993), cited in Long et al. 2003; Supplementary file 1].

We analyzed pupillometry data from the trials that were used in the gaze behavior analysis described above. We pre-processed the pupillometry data (see Supplementary file 1 for details) using the PupillometryR v.0.0.3 R package (Forbes 2020) while carefully checking that the processing did not yield a large artifact. Figure 3.3 shows an example of the time series data during each step of processing. We analyzed approximately the first half of each animation because the apes did not usually persist in viewing the stimuli for the entire duration. Moreover, Morita et al. (2012) observed pupil dilation when participants viewed impossible human movement occurring around 2−3 s from animation onset [Morita et al. (2012) also observed pupil dilation for possible robot movements in a later period, but this is of less relevance to the current study]. As it took approximately 1.5 s to bend both elbows (the point at which the biomechanical violation was most apparent during an impossible movement) and stretch both arms out again, approximately the first half of the animations (approx. 3 s) showed this action sequence twice. For each trial, we downsampled the time series data by averaging data points in ten- (KS) or two-point (KCZ) time-bins. This enabled us to reduce the dataset size while accommodating differences in the number of data points (KS, 300 Hz; KCZ, 60 Hz). We removed trials with > 15% missing values. For baseline correction, we averaged time-bin values corresponding to approximately 300−500 ms from the animation onset and used this as the baseline. We then subtracted it from the following time-bin values. We finally averaged these baseline-corrected values in time-bins corresponding to approximately 2−3 s from an animation's onset. We refer to this output as the "pupil dilation score", with a larger score indicating increased pupil diameter from baseline, and vice versa. The pupil dilation scores were compared between possible and impossible movements for the human and robot animations separately using paired *t* tests.

3.3.2 Results

For the human animations, two individuals were removed from the analysis because their data were missing for either or both conditions (possible/impossible movements). We used data from the second trial instead of the first trial for one case (impossible movement). The screen viewing time did not significantly differ between the possible [median (interquartile range) 3.65 (2.94−4.17)] and impossible movements [4.27 (3.08−5.49)] (Wilcoxon signed-rank test: $n = 14$, $V = 75$, $p = 0.173$). Figure 3.4a shows the proportion of time spent looking at elbows. The proportion of elbow gaze time was not significantly different across the possible [0.01 (0−0.08)] vs. impossible movements [0.12 (0.05−0.27)] [$n = 14$, $V = 73$, $p = 0.057$, Cliff's d = 0.50, 95% CI = (−0.02, 0.81)]. For the robot animations, two individuals were removed from the analysis for the same reason described above. We used data from the second trial for one case (possible movement). The screen viewing time did not significantly differ between the possible [4.25 (3.32−5.91)] and impossible movements [4.69 (3.24−5.50)] (Wilcoxon signed-rank test: $n = 14$, $V = 59$, $p = 0.715$). Again, the proportion of elbow gaze time was not significantly different across the possible [0.07 (0.03−0.10)] vs. impossible movements [0.10 (0.01−0.13)] ($n = 14$, $V = 54$, $p = 0.588$, Cliff's d = 0.21, 95% CI = (−0.29, 0.62)]. Regarding pupillometry, nine and ten participants were removed from the analyses for human and robot animations, respectively, because their data were insufficient. The pupil dilation score was not significantly different between the possible and impossible movements in both the human ($t_6 = 0.79$, $p = 0.460$) and robot animations ($t_5 = 0.31$, $p = 0.772$; for results, see Supplementary file 1, Fig. S3.7).

3.3.3 Discussion

These results did not clearly suggest that the apes understood the biomechanics underlying the arm movements. Although the apes seemed to look at the human elbows for a longer period of time when viewing the impossible movement compared with the possible movement, the difference was not statistically significant. In this experiment, the model was either a heterospecific (human) or unfamiliar agent (robot); we did not include a conspecific agent. Conspecific body images, rather than human body images, may elicit enhanced processing of body structure or action cues (e.g., Gao et al. 2020; Gao and Tomonaga 2020b; Hattori et al. 2010; Kano and Call 2014). Therefore, in experiment 2, we further examined ape understanding of biomechanics using animations of conspecific-like apes.

3.4 Experiment 2

Using the same apparatus as experiment 1, we presented elbow movement in nonhuman great ape animations. We predicted that apes may have a better understanding of conspecific compared with robot and human biomechanics, and hence that they would look at ape elbows for longer when viewing impossible vs. possible movements.

3.4.1 Methods

Fifteen apes (the same participants as in experiment 1, except one fewer ape from KCZ) participated in experiment 2. We used a procedure that was identical to that in experiment 1, except that we used a nonhuman ape animation, created based on a chimpanzee figure, which displayed similar possible and impossible elbow movements (Fig. 3.2c; Supplementary files 3.6, 3.7). A session comprised two trials (i.e., presentation of 6-s animation) so that the participants viewed both animations (i.e., possible and impossible movements) in a session. They underwent two sessions on separate days; data for the second session were missing for two participants at KCZ.

3.4.2 Results

One individual was removed from the analysis because their data were missing. We analyzed the data only from the first trials. The screen viewing time was not significantly different between possible [5.36 (4.93−5.81)] and impossible movements [4.78 (3.61−5.40)] (Wilcoxon signed-rank test: $n = 14$, $V = 30$, $p = 0.173$). Figure 3.4b shows the proportion of time spent looking at elbows. The proportion of elbow gaze time was not significantly different between possible [0.07 (0.02−0.14)] and impossible movements [0.11 (0−0.19)] ($n = 14$, $V = 47$, $p = 0.946$, Cliff's d = 0.07, 95% CI = (−0.41, 0.52)]. Regarding pupillometry, nine participants were also removed from the analysis because their data were insufficient. The pupil dilation score (i.e., pupil diameter change from baseline, averaged approx. 2−3 s from animation onset) was not significantly different between possible and impossible movements (t [4] = 1.58, $p = 0.190$; for results, see Supplementary file 1, Fig. S3.8).

3.4.3 Discussion

Again, the result did not suggest that the apes understood the biomechanics of the movements shown in the ape animations. However, the apes may have perceived the animations as strange or uncanny because of differences between their appearance (texture, color, etc.) and movements (trajectory, speed, acceleration, etc.) and those of real apes [cf. uncanny valley (Mori 1970; Mori et al. 2012)], and hence did not focus on the elbow movements. In a previous study (Steckenfinger and Ghazanfar 2009), for example, long-tailed macaques (*Macaca fascicularis*) differentiated their gaze between realistic CG monkey images compared with real monkey images and unrealistic CG monkey images. Thus, we attempted to reduce the unfamiliar nature of the nonhuman ape animations in experiment 3.

3.5 Experiment 3

We generated a modified ape animation in which arm movements were made while the ape stood quadrupedally. We hypothesized that this posture might be more familiar to apes, and thus, that the revised animations could enable enhanced processing of biomechanics compared with the animations of the bipedally standing apes used in experiment 2 [cf. for the effect of posture familiarity on the inversion effect. see Gao and Tomonaga (2020b)]. We presented the animations of quadrupedally standing apes as well as the human and robot animations (bipedally standing as in experiment 1) to a new group of chimpanzees. As with the participants in experiment 1, the chimpanzees in this experiment were familiar with humans, but not with robots. We predicted that the apes would show clearer signs that they detected biomechanical violations in the ape animations, as indicated by more time spent looking at the elbows when viewing impossible vs. possible movements.

3.5.1 Methods

Ten chimpanzees (seven female, three males; range 19−54 years old, including estimated age) at the Primate Research Institute, Kyoto University (PRI), participated in experiment 3 (Supplementary file 1, Table S3.1). We presented an ape animation in which the ape bent its right elbow either inward or outward while standing quadrupedally (Fig. 3.2d; Supplementary files 3.8, 3.9). We also presented the human and robot animations from experiment 1, in which the elbows were bent while the human or robot stood bipedally. A session comprised six trials, such that the participants viewed all six animations in a session (i.e., ape, human, and robot animations displaying possible and impossible movements). Two sessions were completed on separate days. We did not include sound in the animations, as it seemed to have a minimal impact in the previous experiments. A 4-s attention-getter (two small photographs of chimpanzees presented sequentially with brown noise) preceded each animation. We used a different attention-getter in experiment 3 with the goal of an enhanced control for the baseline pupil diameter. Chimpanzees at PRI participated individually in an indoor experimental booth (1.8 × 2.15 × 1.75 m) and viewed the monitor through a transparent 2-cm-thick acrylic panel. The other procedures were identical to those in experiment 1.

3.5.2 Results

For the ape animations, no individuals were removed from the analysis, and data from only the first trials were analyzed. The screen viewing time was not significantly different between the possible [5.58 (5.04−5.69)] and impossible movements [5.59 (4.28−5.97)] (Wilcoxon signed-rank test: $n = 10$, $V = 27$, $p = 1$). Figure 3.5 shows the proportion of time spent looking at elbows. The proportion of elbow gaze time was not significantly different between the possible [0 (0−0.06)] and impossible movements [0 (0−0.07)] ($n = 10$, $V = 9$, $p = 0.844$, Cliff's d = 0, 95% CI = (−0.45, 0.45)]. For the human animations, one individual was removed from the analysis because their data for one condition were missing. We analyzed data from the second trials for three cases (two for possible and one for impossible movements). Screen viewing time was not significantly different between the possible [5.89

(4.24−5.94)] and impossible movements [5.65 (3.43−5.78)] (Wilcoxon signed-rank test: $n = 9$, $V = 15$, $p = 0.426$). The proportion of elbow gaze time was not significantly different between the possible [0.14 (0.03−0.17)] and impossible movements [0.12 (0.06−0.29)] ($n = 9$, $V = 30$, $p = 0.426$, Cliff's d = 0.33, 95% CI = (−0.32, 0.77)]. For the robot animation, no individuals were removed from the analysis, and only data from the first trials were analyzed. The screen viewing time did not significantly differ between the possible [4.33 (3.72−5.86)] and impossible movements [5.62 (3.99−5.99)] (Wilcoxon signed-rank test: $n = 10$, $V = 35$, $p = 0.492$). Intriguingly, the proportion of elbow gaze time was significantly higher when the apes viewed impossible [0.17 (0.12−0.26)] vs. possible movements [0.08 (0.04−0.12)] ($n = 10$, $V = 52$, $p = 0.010$, Cliff's d = 0.80, 95% CI = (0.15, 0.97)]. Regarding pupillometry, three, two, and one participants were further removed from the analysis for ape, human, and robot animations, respectively, because their data were insufficient. The pupil dilation score (i.e., pupil diameter change from baseline, averaged approx. 2−3 s from animation onset) was not significantly different between the possible and impossible movements for all animations (ape, $t_6 = 0.38$, $p = 0.719$; human, $t_6 = -0.77$, $p = 0.470$; robot, $t_8 = 0.89$, $p = 0.397$).

3.5.3 Discussion

The chimpanzees in experiment 3 did not differentiate gaze behaviors between possible and impossible human or ape movements. Thus, these results did not suggest that the chimpanzees understood the biomechanics underlying human and ape movements. In contrast, the chimpanzees looked at elbows for longer when viewing impossible vs. possible robot movements. Because the chimpanzees were not expected to be familiar with robotic agents, it is unlikely that they differentiated gaze behaviors based on past experiences. Instead, this result may indicate that, like humans (Morita et al. 2012), chimpanzees can extend some knowledge regarding biomechanics to a novel robot agent. This interpretation is apparently at odds with the finding that the chimpanzees did not distinguish possible and impossible movements in the human and ape animations. This discrepancy may in part be due to the uncanny nature of the CG animations. Specifically, the chimpanzees may have perceived the ape and human animations as uncanny because, while those agents looked like familiar animals, either conspecific or heterospecific, they were dissimilar in some way, such as in their surface texture. Consequently, the apes may have just visually explored the human and ape agents per se, rather than fixating on the elbows. In contrast, the robot with its object-like appearance was not likely perceived as uncanny, and so it may have been easier for the chimpanzees to attend to its movements rather than the agent per se. This view is supported particularly for the ape animation by the results that four of ten participants did not fixate on the ape elbows when viewing both possible and impossible movements, while participants did look at the elbows when viewing at least one of the possible or impossible movements in the human and robot animations.

3.6 Meta-Analysis

Overall, the apes did not look at the elbows for a longer period of time when viewing impossible vs. possible movements, except in the case of the chimpanzees in experiment 3 when viewing the robot animations. Despite the similar stimuli and procedures, experiments 1 and 3 yielded different results for the robot animation. Moreover, the apes in experiment 1 seemed to be able to distinguish the human possible and impossible movements (although this was not statistically significant; see Fig. 3.4a), while this did not seem to be the case for apes participating in experiment 3 (Fig. 3.5). To synthesize the results of the human and robot animations in experiments 1 and 3, we conducted a meta-analysis. Note that this was not possible for the ape animations because different animations were used in experiments 2 and 3 (i.e., standing bipedally/quadrupedally).

We used Cliff's d and a variance estimate for each animation (human and robot) in each experiment (experiments 1 and 3), and ran random effect models (restricted maximum likelihood estimation) using the metaphor v.2.4.0 R package (Viechtbauer 2010). We used an unweighted Cliff's d, which did not give a different weight to each experiment, because it can perform better than a weighted one (Hess et al. 2005; Kromrey et al. 2005). We calculated CIs for the pooled effect using the estimated effect and its variance (Supplementary file 1), and inferred its significance based on whether the CIs included 0. In performing the analysis, we referred to Kim et al. (2018) and adopted the associated R codes for calculation and generation of the forest plot from Errington et al. (2018) and Iorns et al. (2019).

Figure 3.6 shows Cliff's d with the corresponding CI for each of experiments 1 and 3, as well as for the random effect model. CIs of the pooled effect included 0 for both the human [estimated effect: 0.42, 95% CI = (−0.02, 0.72)] and robot animations [estimated effect: 0.51, 95% CI = (−0.15, 0.85)]. This indicates that the difference in gaze behavior between possible and impossible movements was not significant when the two experiments were combined.

3.7 General Discussion

In the present study, we examined the way in which bonobos and chimpanzees understand biomechanics using a series of eye-tracking experiments based on a previous study in human adults and infants (Morita et al. 2012). Specifically, we presented CG animations adapted from the human study to apes, and measured their gaze while they viewed the animations via eye-tracking technology. Morita et al. (2012) found that 12-month-old infants and adults looked at elbows for longer when they were shown biomechanically impossible arm movements compared with possible movements. This pattern was observed both when the movements were displayed by a human and when displayed by a robot. In experiment 1, apes were shown human and robot animations adapted from Morita et al. (2012). Although we found no clear differences in gaze behaviors between possible and impossible movements in either the human or robot animations, the apes showed slight signs that they could distinguish these movements in the human animations. In experiment 2, we presented bipedally standing ape animations. Yet again, the apes did not differentiate their gaze behaviors between possible and impossible movements. Finally, in experiment 3, we continued the line of inquiry from the prior experiments by presenting another group of chimpanzees with modified ape animations in which the apes stood quadrupedally, and hence had a more typical posture, as well as the human and robot animations used in experiment 1. The chimpanzees did not differentiate their gaze behaviors between possible and impossible movements in the human and ape animations. However, they did look at elbows for longer when viewing impossible vs. possible robot movements, as if they had some understanding of robot biomechanics-like properties. The reason why the apes in experiment 3 differentiated their gaze behaviors, while those in experiment 1 did not, is unclear. In the present study, apes were not particularly required or motivated to look carefully at the animations. Thus, some apes might not have specifically fixated on the elbows during the trials because of inattention or restlessness, although we set the minimum inclusion criterion based on screen viewing time. Relatedly, the chimpanzees in experiment 3 seemed to view the stimuli for slightly longer than the apes in experiment 1 (see Supplementary file 1, Figs. S3.1, S3.3), which indicates that different levels of attention paid to stimuli may have affected the results. Alternatively, the chimpanzees in experiment 3 (at PRI) might have had more general experience of observing artificial objects compared with the apes in experiment 1 (at KS and KCZ), which could have indirectly helped the former to distinguish between possible and impossible movements in the robot animations.

Given these mixed results, it is difficult to infer the extent to which apes understand biomechanics. These results could indicate that, unlike humans (e.g., Morita et al. 2012), apes have little or no understanding of biomechanics. However, it is equally plausible that the null results are entirely related to the methodological features of the present study, in particular the CG animations of arm movements. First, the uncanny nature of the CG animations may have superseded the saliency of the impossible movements. Thus, future studies should carefully assess ape perception of CG animations, which could be perceived as strange looking by apes, prior to testing (Chouinard-Thuly et al. 2017). Presentation of a familiarization video prior to the presentation of test stimuli (Reid et al. 2005) or a habituation-dishabituation technique [for related human infant studies, see Christie and Slaughter (2010) and Southgate et al. (2008)] might also help familiarize participants with the experimental stimuli. Second, while we used animations depicting arm movements, animations of other body parts (e.g., faces, hands, or legs) might be more suitable for presentation to apes. For example, human infants spend a lot of time looking at their hands (White et al. 1964) and can regulate their hand movements voluntarily so that their hand enters their visual field (van der Meer 1997; van der Meer et al. 1995). When toddlers manipulate objects, they often watch their hands or those of a social partner (Yoshida and Smith 2008). Thus, hands appear to be especially salient for humans (Geangu et al. 2015; Longhi et al. 2015; Senna et al. 2017), and possible vs. impossible hand movements can be discerned at a young age [at 6 but not at 4 months old (Geangu et al. 2015); several days after birth (Longhi et al. 2015); at 9 months old, but only in those who could perform a pincer grip (Senna et al. 2017)]. This discrimination occurred slightly later in Morita et al. (2012), who examined reactions to elbow movements [i.e., at 12 but not 9 months old; but for a different result, see Morita et al. (2010)]. Compared with elbow movements, hand movements might be more familiar and salient for apes as well. Third, while our stimuli depicted a purposeless, intransitive arm-bending movement, other types of movements (e.g., walking or climbing) or goal-directed actions (e.g., grasping an object) coupled with more extreme examples of biomechanical violation might have elicited a different response from the apes. For example, Reid et al. (2005) reported that 8-month-old infants differentiated biologically possible and impossible arm movements displayed during a reaching action, although this was only the case for infants with certain fine motor skills. Goal-directed actions may be more salient [for a study with human neonates, see Craighero et al. (2011)], and thus serve to elicit differentiated gaze behaviors between conditions. Furthermore, an early group of experiments yielded roughly converging findings indicating that the mirror-like motor resonance system in humans may be more or less activated by the observation of both intransitive movements and goal-directed actions, whereas such a system in macaque monkeys may only be activated by goal-directed actions, or perhaps only very subtly by the observation of intransitive movements (reviewed by Rizzolatti et al. 2014; Rizzolatti and Craighero 2004; Rizzolatti and Sinigaglia 2010). More recently, this view has been challenged by data indicating that so-called mirror neurons in monkeys are also, to some extent, activated via the observation of intransitive movements (Kraskov et al. 2009; Papadourakis and Raos 2017, 2019). Nevertheless, it is possible to speculate that nonhuman and human animals might differ in terms of reactivity to intransitive movements relative to that to goal-directed actions. Although further research is needed, this possibility is well in line with the overall null results of the present study. Therefore, movies depicting movement of more salient body parts such as the hands (Geangu et al. 2015; Longhi et al. 2015; Senna et al. 2017), or goal-directed actions (Geangu et al. 2015; Reid et al. 2005), might facilitate research regarding how apes perceive violations of biomechanics.

We do not expect the recording accuracy of the ape eye-tracking technology to have a profound impact on the results. We did not quantitatively measure the error and could not compare it with that in the previous study on humans (Morita et al. 2012). However, we closely followed procedures that have been established through previous ape eye-tracking studies (reviewed in Hopper et al. 2020). Experiments have shown that these procedures can enable reliable recording of ape gaze with recording errors of less than 1° (Kano and Tomonaga 2009; Kano et al. 2011; Hirata et al. 2010). Although there are also some differences in procedures between those studies and the present one (e.g., the use of juice), Kano and Tomonaga (2009), for example, reported comparable levels of error between chimpanzee and human participants of less than 0.5°. Kano et al. (2011) also noted that the error was about 0.5°−0.7° for all participant groups, including nonhuman and human apes. Notably, some of the chimpanzees from those studies also participated in the present study, namely, chimpanzees at KS and PRI. The error might be larger for several participants, in particular chimpanzees at KCZ, as we noted above (*Stimuli and Procedure* in *Methods*). Thus, our results should be interpreted with caution. However, those participants accounted for a small proportion of the participant group in each experiment, and we even omitted some of their data from statistical analyses because they spent an insufficient amount of time viewing the screen. Thus, it is unlikely that the data from those individuals had a pronounced impact on the overall results. The relatively small size of areas of interest for elbows might affect the nuanced results of experiment 1. Relatedly, a potential limitation of the present study is that we did not consider in depth the role played by peripheral vision (Orquin and Holmqvist 2018). Stimuli depicting a body at closer range might be useful for the clearer differentiation of gazes directed at different body parts. However, our findings suggest that the arm movements of our stimuli were sufficiently salient to cause the apes to orient their gaze accordingly. In experiment 3, the chimpanzees looked at the robot elbows for longer when viewing impossible vs. possible movements. Remarkably, the screen viewing time was not significantly different between those conditions. Thus, the eye-tracking experiments were able to capture finely differentiated gaze behaviors. This underlines the chief advantage of non-invasive ape eye tracking, namely, a prominent spatiotemporal resolution that is difficult to achieve by manual coding of gaze or head orientation (Hopper et al. 2020; Kano and Call 2017).

Overall, we did not observe pupil dilation when the apes viewed impossible movements. The present study used realistic, graphically rich animations; however, we did not control for the effects of specific visual properties within the animation such as brightness and contrast, which could modulate pupil diameter (Bradley et al. 2017). As these could hamper the detection of pupil dilation associated with affective responses, the pupillometry results should be interpreted with caution. Nonetheless, at the very least, our study suggests that the measurement of ape pupil diameter is feasible, and we believe that detailed data visualization (Supplementary file 1, Figs. S3.10−S3.18) might be instrumental for future studies.

In conclusion, the findings of the present study, in which apes underwent eye tracking whilst watching CG animations, add to those of previous work on primates' visual processing of movements made by other animals. Specifically, we collected data on ape behavior via a similar method to that used in human developmental psychology research (Morita et al. 2012). Some of our results indicate that some apes may have an understanding of biomechanics that they can draw on to infer the movements of a novel robotic agent. However, the null hypothesis regarding gaze behavior and pupil response was not reject. Thus, conclusive evidence regarding the extent to which apes understand biomechanics was not obtained in the current study. Nevertheless, the results of this study have implications for the design of nonhuman animal studies involving gaze behavior, pupillometry, and/or CG animations.

3.8 Acknowledgments

We would like to especially thank Mr. H. Ishimoto and Mr. K. Nagamachi at the Department of Computer Science and Engineering, Toyohashi University of Technology, for making the high-quality 3D animations. Further, we are grateful to Dr. N. Morimura, Dr. F. Kano, and all the staff at KS; Ms. H. Bando, Mr. M. Sato, Dr. Y. Yamanashi, and Dr. M.

Tanaka at KCZ; and Ms. E. Ichino, Dr. Y. Kawaguchi, and members of the Language & Intelligence section of the PRI, for their kind help in conducting the current study. We also thank Dr. T. Matsuzawa for the financial support. We thank Sydney Koke, MFA, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript. Finally, we are grateful to the editor and anonymous reviewers for their constructive feedback during the review process.

3.9 References

Avenanti A, Bolognini N, Maravita A, Aglioti SM (2007) Somatic and motor components of action simulation. Curr Biol 17(24):2129–2135. https://doi.org/10.1016/j.cub.2007.11.045

Bardi L, Regolin L, Simion F (2011) Biological motion preference in humans at birth: role of dynamic and configural properties. Dev Sci 14(2):353–359. https://doi.org/10.1111/j.1467-7687.2010.00985.x

Bardi L, Regolin L, Simion F (2014) The first time ever I saw your feet: inversion effect in newborns' sensitivity to biological motion. Dev Psychol 50(4):986–993. https://doi.org/10.1037/a0034678

Bidet-Ildei C, Kitromilides E, Orliaguet J-P, et al (2014) Preference for point-light human biological motion in newborns: contribution of translational displacement. Dev Psychol 50(1):113–120. https://doi.org/10.1037/a0032956

Blake R (1993) Cats perceive biological motion. Psychol Sci 4(1):54–57. https://doi.org/10.1111/j.1467-9280.1993.tb00557.x

Bradley MM, Sapigao RG, Lang PJ (2017) Sympathetic ANS modulation of pupil diameter in emotional scene perception: effects of hedonic content, brightness, and contrast. Psychophysiology 54(10):1419–1435. https://doi.org/10.1111/psyp.12890

Campbell MW, Carter JD, Proctor D et al (2009) Computer animations stimulate contagious yawning in chimpanzees. Proc Royal Soc B Biol Sci 276(1676):4255–4259. https://doi.org/10.1098/rspb.2009.1087

Chouinard-Thuly L, Gierszewski S, Rosenthal GG, et al (2017) Technical and conceptual considerations for using animated stimuli in studies of animal behavior. Curr Zool 63(1):5–19. https://doi.org/10.1093/cz/zow104

Christie T, Slaughter V (2010) Movement contributes to infants' recognition of the human form. Cognition 114:329–337. https://doi.org/10.1016/j.cognition.2009.10.004

Cliff N (1993) Dominance statistics: ordinal analyses to answer ordinal questions. Psychol Bull 114(3):494–509. https://doi.org/10.1037/0033-2909.114.3.494

Costantini M, Galati G, Ferretti A, et al (2005) Neural systems underlying observation of humanly impossible movements: an fMRI study. Cereb Cortex 15(11):1761–1767. https://doi.org/10.1093/cercor/bhi053

Craighero L, Leo I, Umiltà C, Simion F (2011) Newborns' preference for goal-directed actions. Cognition 120(1):26–32. https://doi.org/10.1016/j.cognition.2011.02.011

De Agrò M, Rößler DC, Kim K, Shamble PS (2021) Perception of biological motion in point-light displays by jumping spiders. bioRxiv. https://doi.org/10.1101/2021.02.24.432515

Errington TM, Tan FE, Lomax J et al (2018) Replication studies. Retrieved from osf.io/p7ayb

Feng D, Cliff N (1993) Comparisons of power and size of *d* and *t* statistics under normal and nonnormal assumptions. University of Southern California, Los Angeles (Unpublished manuscript)

Forbes SH (2020) PupillometryR: an R package for preparing and analysing pupillometry data. J Open Source Software 5(50):2285. https://doi.org/10.21105/joss.02285

Gao J, Tomonaga M (2020a) Body perception in chimpanzees (*Pan troglodytes*): the effect of body structure changes. J Comp Psychol 134(2):222–231. https://doi.org/10.1037/com0000214

Gao J, Tomonaga M (2020b) How chimpanzees and children perceive other species' bodies: comparing the expert effect. Dev Sci 23(6):e12975. https://doi.org/10.1111/desc.12975

Gao J, Kawakami F, Tomonaga M (2020) Body perception in chimpanzees and humans: the expert effect. Sci Rep 10:7148. https://doi.org/10.1038/s41598-020-63876-x

Geangu E, Senna I, Croci E, Turati C (2015) The effect of biomechanical properties of motion on infants' perception of goal-directed grasping actions. J Exp Child Psychol 129:55–67. https://doi.org/10.1016/j.jecp.2014.08.005

Hattori Y, Kano F, Tomonaga M (2010) Differential sensitivity to conspecific and allospecific cues in chimpanzees and humans: a comparative eye-tracking study. Biol Let 6(5):610–613. https://doi.org/10.1098/rsbl.2010.0120

Hepach R, Vaish A, Kano F, Albiach-Serrano A, Benziad L, Call J, Tomasello M (2021) Chimpanzees' (*Pan troglodytes*) internal arousal remains elevated if they cannot themselves help a conspecific. J Comp Psychol 135(2):196–207. https://doi.org/10.1037/com0000255

Hess MR, Kromrey JD, Ferron JM et al (2005) Robust inference in meta-analysis: an empirical comparison of point and interval estimates using the standardized mean difference and cliff's delta. Annual Meeting of the American Educational Research Association. Montreal, pp 11−15

Hirata S, Fuwa K, Sugama K, et al (2010) Facial perception of conspecifics: chimpanzees (*Pan troglodytes*) preferentially attend to proper orientation and open eyes. Anim Cogn 13(5):679–688. https://doi.org/10.1007/s10071-010-0316-y

Hopper LM, Gulli RA, Howard LH, et al (2020) The application of noninvasive, restraint-free eye-tracking methods for use with nonhuman primates. Behav Res 53(3):1003–1030. https://doi.org/10.3758/s13428-020-01465-6

Hothorn T, Hornik K (2019) exactRankTests: exact distribution for rank and permutation tests. R package version 0.8–31. https://CRAN.R-project.org/package=exactRankTests

Iacoboni M, Woods RP, Brass M, et al (1999) Cortical mechanisms of human imitation. Science 286(5449):2526–2528. https://doi.org/10.1126/science.286.5449.2526

Iorns E, Tsui R, Denis A et al (2019) Meta-analysis. Retrieved from osf.io/c69jx

Johansson G (1973) Visual perception of biological motion and a model for its analysis. Percept Psychophys 14(2):201–211. https://doi.org/10.3758/BF03212378

Kano F, Call J (2014) Cross-species variation in gaze following and conspecific preference among great apes, human infants and adults. Anim Behav 91:137–150. https://doi.org/10.1016/j.anbehav.2014.03.011

Kano F, Call J (2017) Great ape social attention. In: Watanabe S, Hofman M, Shimizu T (eds) Evolution of the Brain, Cognition, and Emotion in Vertebrates. Brain Science. Springer, Tokyo, pp 187–206. https://doi.org/10.1007/978-4-431-56559-8_9

Kano F, Tomonaga M (2009) How chimpanzees look at pictures: a comparative eye-tracking study. Proc Royal Soc B Biol Sci 276(1664):1949–1955. http://doi.org/10.1098/rspb.2008.1811

Kano F, Hirata S, Call J, Tomonaga M (2011) The visual strategy specific to humans among hominids: a study using the gap-overlap paradigm. Vis Res 51(23–24):2348–2355. https://doi.org/10.1016/J.VISRES.2011.09.006

Kim J, Afshari A, Sengupta R et al (2018) Replication study: melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. eLife 7:e39944. https://doi.org/10.7554/eLife.39944

Komori N, Katayama J, Kitazaki M, Itakura S (2006) Do infants prefer possible human movements? The 5th International Conference of the Cognitive Science. Vancouver, pp 143–144

Kraskov A, Dancause N, Quallo MM, et al (2009) Corticospinal neurons in macaque ventral premotor cortex with mirror properties: a potential mechanism for action suppression? Neuron 64(6):922–930. https://doi.org/10.1016/j.neuron.2009.12.010

Kret ME, Prochazkova E, Sterck EHM, Clay Z (2020) Emotional expressions in human and non-human great apes. Neurosci Biobehav Rev 115:378–395. https://doi.org/10.1016/j.neubiorev.2020.01.027

Kromrey JD, Hogarty KY, Ferron JM et al (2005) Robustness in meta-analysis: an empirical comparison of point and interval estimates of standardized mean differences and Cliff’s delta. Joint Statistical Meetings. Minneapolis, pp 7−11

Long JD, Feng D, Cliff N (2003) Ordinal analysis of behavioral data. In: Schinka JA, Velicer WF (eds) Handbook of psychology: research methods in psychology, vol 2. Wiley, Hoboken, pp 635–661. https://doi.org/10.1002/0471264385.wei0225

Longhi E, Senna I, Bolognini N et al (2015) Discrimination of biomechanically possible and impossible hand movements at birth. Child Dev 86(2):632–641. https://doi.org/10.1111/cdev.12329

Massen JJM, Behrens F, Martin JS et al (2019) A comparative approach to affect and cooperation. Neurosci Biobehav Rev 107:370–387. https://doi.org/10.1016/j.neubiorev.2019.09.027

Matsuno T, Fujita K (2018) Body inversion effect in monkeys. PLoS ONE 13(10):e0204353. https://doi.org/10.1371/journal.pone.0204353

Mori M (1970) Bukimi no tani (the uncanny valley). Energy 7(4):33–35.

Mori M, MacDorman KF, Kageki N (2012) The uncanny valley (from the field). IEEE Robot Autom Magaz 19(2):98–100. https://doi.org/10.1109/MRA.2012.2192811

Morita T, Katayama N, Kitazaki M, Itakura S (2010) Development of perception of human and robot body movement. J Robot Soc Japan 28(4):463–469. https://doi.org/10.7210/jrsj.28.463

Morita T, Slaughter V, Katayama N, et al (2012) Infant and adult perceptions of possible and impossible body movements: an eye-tracking study. J Exp Child Psychol 113(3):401–414. https://doi.org/10.1016/j.jecp.2012.07.003

Nakayasu T, Watanabe E (2014) Biological motion stimuli are attractive to medaka fish. Anim Cogn 17(3):559–575. https://doi.org/10.1007/s10071-013-0687-y

Nakayasu T, Yasugi M, Shiraishi S et al (2017) Three-dimensional computer graphic animations for studying social approach behaviour in medaka fish: effects of systematic manipulation of morphological and motion cues. PLoS ONE 12(4):e0175059. https://doi.org/10.1371/journal.pone.0175059

Orquin JL, Holmqvist K (2018) Threats to the validity of eye-movement research in psychology. Behav Res 50(4):1645–1656. https://doi.org/10.3758/s13428-017-0998-z

Papadourakis V, Raos V (2017) Evidence for the representation of movement kinematics in the discharge of F5 mirror neurons during the observation of transitive and intransitive actions. J Neurophysiol 118(6):3215–3229. https://doi.org/10.1152/jn.00816.2016

Papadourakis V, Raos V (2019) Neurons in the macaque dorsal premotor cortex respond to execution and observation of actions. Cereb Cortex 29(10):4223–4237. https://doi.org/10.1093/cercor/bhy304

Peinkhofer C, Knudsen GM, Moretti R, Kondziella D (2019) Cortical modulation of pupillary function: systematic review. PeerJ 7:e6882. https://doi.org/10.7717/peerj.6882

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Regolin L, Tommasi L, Vallortigara G (2000) Visual perception of biological motion in newly hatched chicks as revealed by an imprinting procedure. Anim Cogn 3(1):53–60. https://doi.org/10.1007/s100710050050

Reid V, Belsky J, Johnson M (2005) Infant perception of human action: toward a developmental cognitive neuroscience of individual differences. Cogn Brain Behav 9(3):193–210.

Reid VM, Hoehl S, Landt J, Striano T (2008) Human infants dissociate structural and dynamic information in biological motion: evidence from neural systems. Soc Cogn Affect Neurosci 3(2):161–167. https://doi.org/10.1093/scan/nsn008

Riečanský I, Lamm C (2019) The role of sensorimotor processes in pain empathy. Brain Topogr 32(6):965–976. https://doi.org/10.1007/s10548-019-00738-4

Rizzolatti G, Craighero L (2004) The mirror-neuron system. Annu Rev Neurosci 27:169–192. https://doi.org/10.1146/annurev.neuro.27.070203.144230

Rizzolatti G, Sinigaglia C (2010) The functional role of the parieto-frontal mirror circuit: interpretations and misinterpretations. Nat Rev Neurosci 11(4):264–274. https://doi.org/10.1038/nrn2805

Rizzolatti G, Cattaneo L, Fabbri-Destro M, Rozzi S (2014) Cortical mechanisms underlying the organization of goal-directed actions and mirror neuron-based action understanding. Physiol Rev 94(2):655–706. https://doi.org/10.1152/physrev.00009.2013

Rogmann JJ (2013) orddom: ordinal dominance statistics. R package version 3.1. https://CRAN.R-project.org/package=orddom

Romani M, Cesari P, Urgesi C, et al (2005) Motor facilitation of the human cortico-spinal system during observation of bio-mechanically impossible movements. Neuroimage 26(3):755–763. https://doi.org/10.1016/j.neuroimage.2005.02.027

Senna I, Addabbo M, Bolognini N et al (2017) Infants' visual recognition of pincer grip emerges between 9 and 12 months of age. Infancy 22(3):389–402. https://doi.org/10.1111/infa.12163

Simion F, Regolin L, Bulf H (2008) A predisposition for biological motion in the newborn baby. Proc Natl Acad Sci USA 105(2):809–813. https://doi.org/10.1073/pnas.0707021105

Southgate V, Johnson MH, Csibra G (2008) Infants attribute goals even to biomechanically impossible actions. Cognition 107(3):1059–1069. https://doi.org/10.1016/j.cognition.2007.10.002

Steckenfinger SA, Ghazanfar AA (2009) Monkey visual behavior falls into the uncanny valley. Proc Natl Acad Sci USA 106(43):18362–18366. https://doi.org/10.1073/pnas.0910063106

Tomonaga M (2001) Visual search for biological motion patterns in chimpanzees (*Pan troglodytes*). Psychologia 44(1):46–59

Vallortigara G, Regolin L (2006) Gravity bias in the interpretation of biological motion by inexperienced chicks. Curr Biol 16(8):R279–R280. https://doi.org/10.1016/j.cub.2006.03.052

Vallortigara G, Regolin L, Marconato F (2005) Visually inexperienced chicks exhibit spontaneous preference for biological motion patterns. PLoS Biol 3(7):e208. https://doi.org/10.1371/journal.pbio.0030208 van der Meer AL (1997) Keeping the arm in the limelight: advanced visual control of arm movements in neonates. Eur J Paediatr Neurol 1(4):103–108. https://doi.org/10.1016/S1090-3798(97)80040-2

van der Meer AL, van der Weel FR, Lee DN (1995) The functional significance of arm movements in neonates. Science 267(5198):693–695. https://doi.org/10.1126/science.7839147

Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. J Stat Soft 36(3):1–48. https://doi.org/10.18637/jss.v036.i03

Watanabe S, Troje NF (2006) Towards a “virtual pigeon”: a new technique for investigating avian social perception. Anim Cogn 9(4):271–279. https://doi.org/10.1007/s10071-006-0048-1

White B, Castle P, Held R (1964) Observations on the development of visually-directed reaching. Child Dev 35(2):349–364. https://doi.org/10.2307/1126701

Yoshida H, Smith LB (2008) What’s in view for toddlers? Using a head camera to study visual experience. Infancy 13(3):229–248. https://doi.org/10.1080/15250000802004437

3.10 Figures

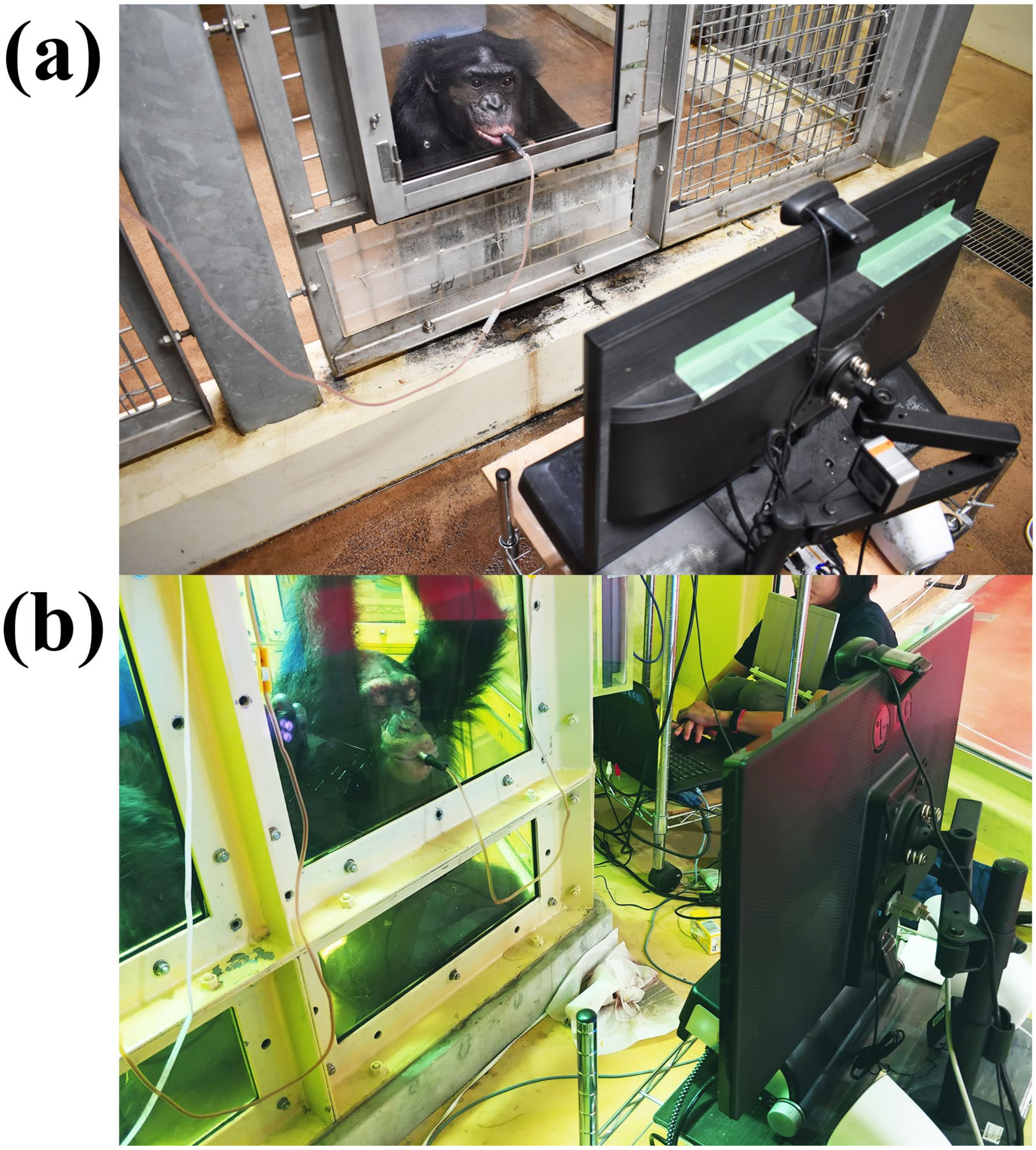

Fig. 3.1 Experimental settings at **a** Kumamoto Sanctuary and **b** at Kyoto City Zoo.

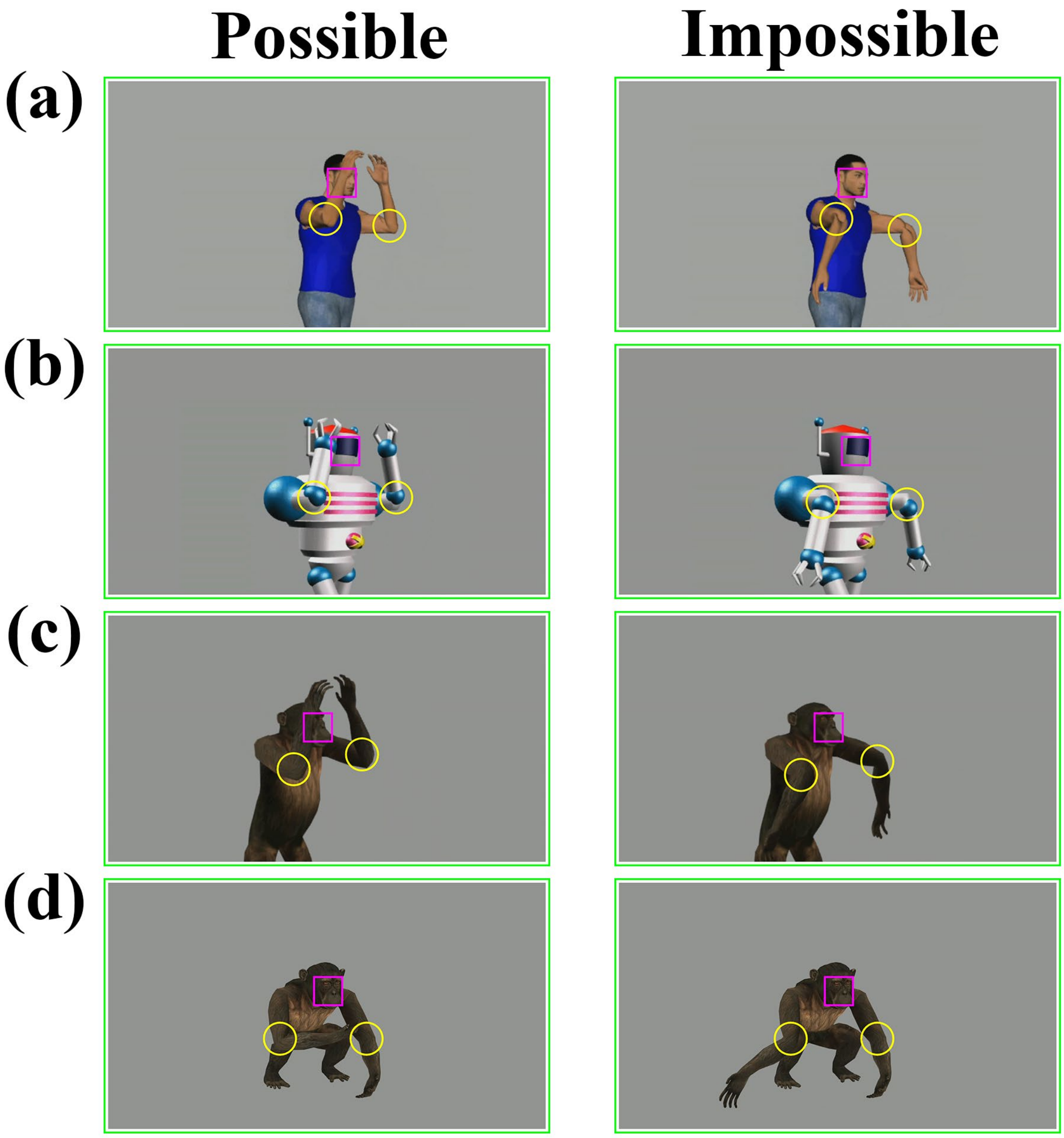

Fig. 3.2 Representative stills of animation stimuli: **a** human, **b** robot, **c** biped ape, and **d** quadruped ape. *Left* Possible elbow movements, *right* impossible elbow movements. *Circles* Areas of interest (AOI) for elbows, *squares* AOI for face (analysis of gaze time directed at face was not reported), *rectangles* AOIs for screen. These AOIs were not included in the stimulus animations. In the human, robot, and biped ape animations, the position of the whole body on the screen moved according to the elbow movements, unlike in Morita et al. (2012). Thus, the AOIs for elbows and face moved to track the corresponding body parts throughout the animation.

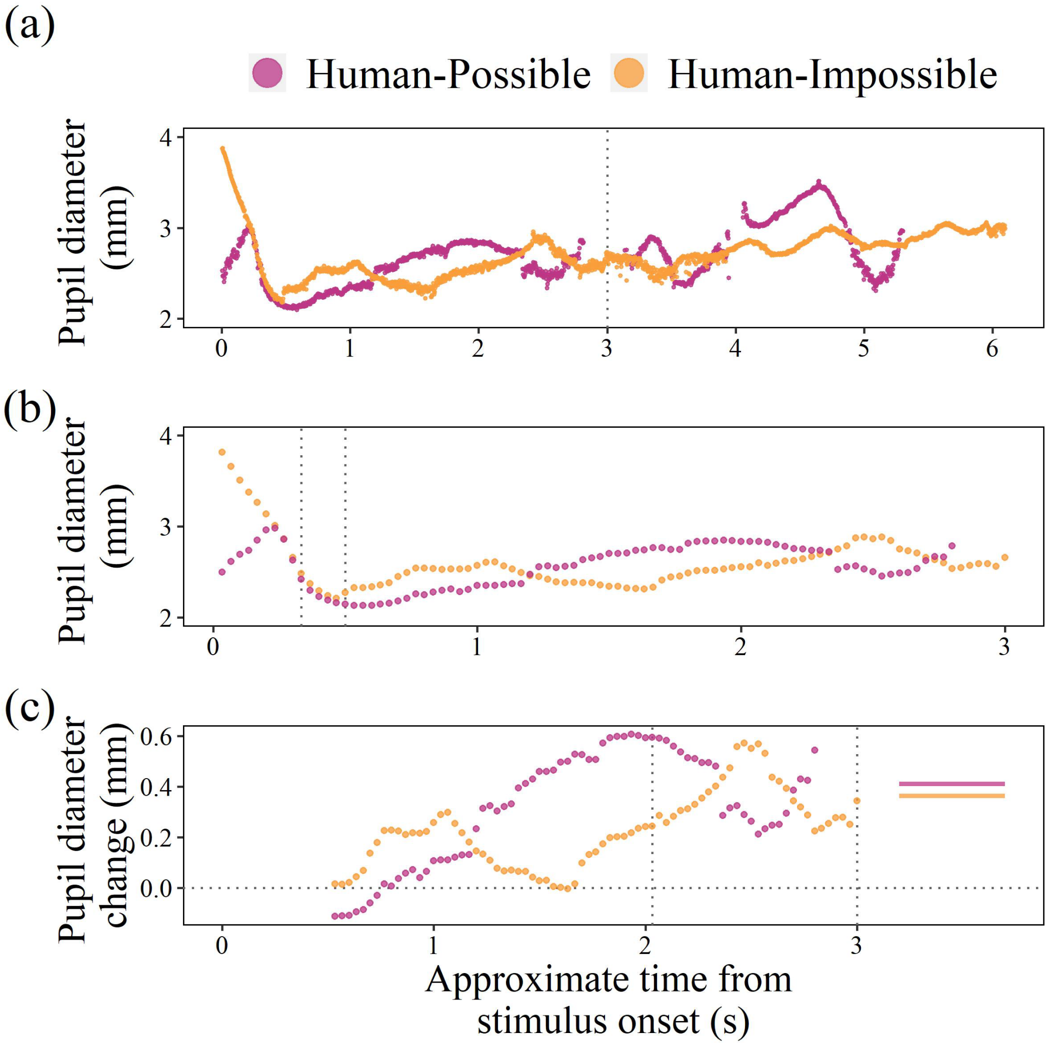

Fig. 3.3 **a** Example of pupillometry data from one individual when viewing the human animations. For visual comparison, two time series datasets were plotted simultaneously. We first averaged pupil diameters across the right and left eyes. Approximately the first half of the data, corresponding to the *left* of the *vertical dotted line*, were analyzed in the following step. **b** We downsampled the time series data by averaging the data points in time-bins. The *area between the vertical dotted line* (approx. 300−500 ms from animation onset) served as the baseline in the following step. **c** The values were then baseline corrected. The *horizontal lines* show the averaged values approximately 2−3 s from the stimulus onset (referred to as the "pupil dilation score" in the main text), which were subsequently analyzed using paired *t* tests. Note that the axis scales differ between the plots. Similar plots for each individual are provided in Supplementary file 1.

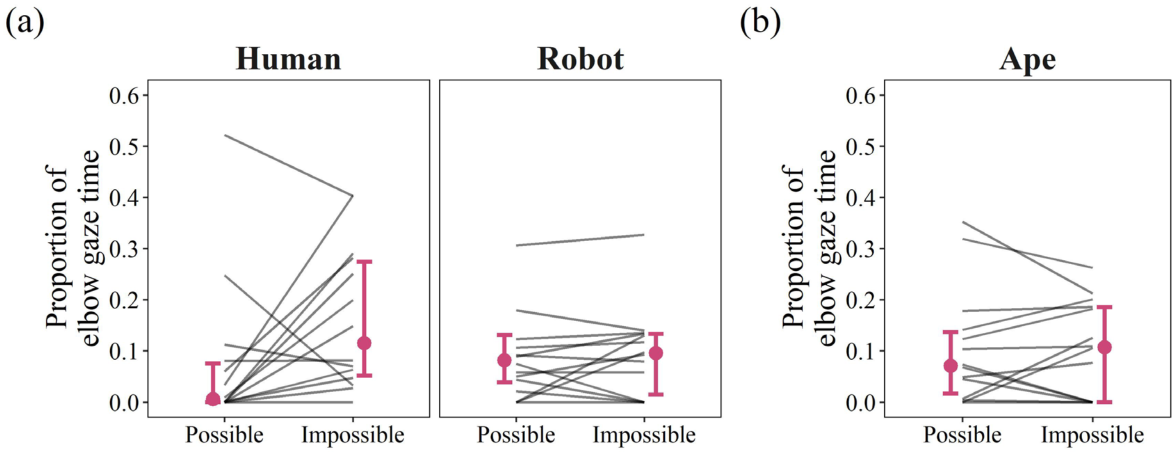

Fig. 3.4 **a** Proportions of time spent looking at elbows when viewing the human and robot animations (experiment 1). **b** Proportions of time spent looking at elbows when viewing the biped ape animations (experiment 2). *Dots* show the median, *error bars* show the first and third quantiles.

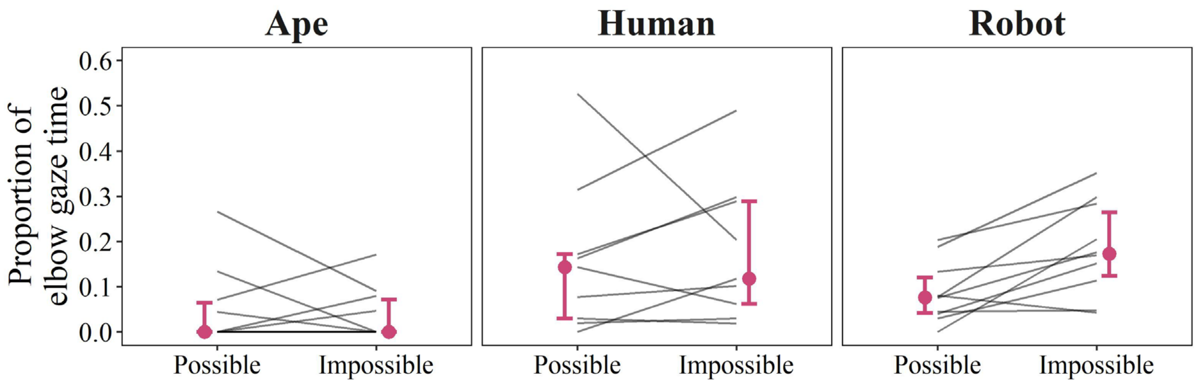

Fig. 3.5 Proportions of time spent looking at elbows when viewing human, robot, and quadruped ape animations (experiment 3). *Dots* show the median, *error bars* show the first and third quantiles.

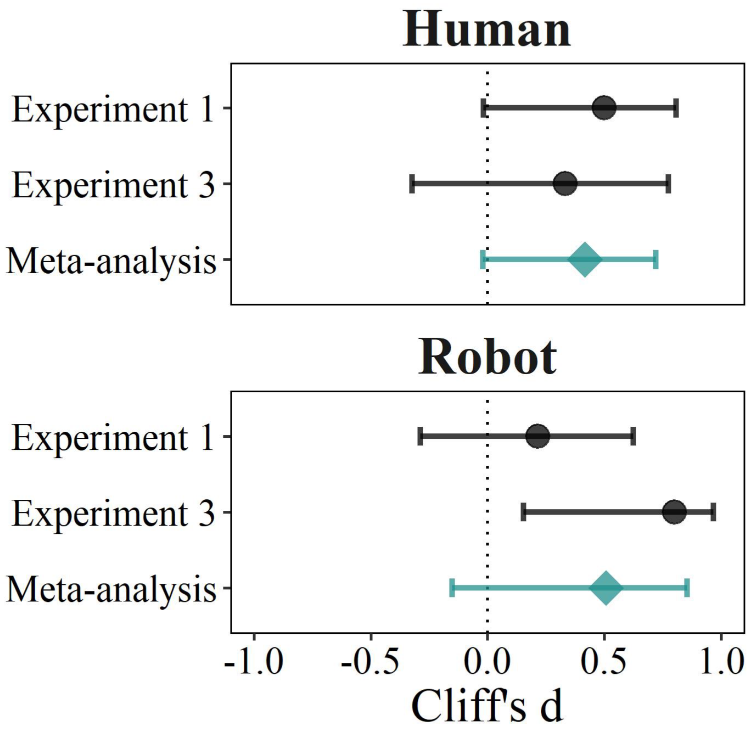

Fig. 3.6 Forest plot showing the results of the meta-analysis. *Upper panels* Results for the human animations, *lower panels* results for the robot animations. *Dots* show Cliff’s d, *error bars* show the corresponding 95% confidence intervals.

3.11 Supplementary Materials

Supplementary file 1

Supplementary Methods

Table S3.1 Ape participants at Kumamoto Sanctuary (KS), Kyoto City Zoo (KCZ), and the Primate Research Institute (PRI).

Species	Site	Name	Age	Sex	GAIN ID[b]
Bonobos	KS	Ikela	27	F	0008
		Junior	24	M	0009
		Connie-Lenore	37	F	0006
		Lolita	29	F	0007
		Louise	46	F	0010
		Vijay	15	M	0011
Chimpanzees	KS	Hatsuka	10	F	0704
		Iroha	10	F	0708
		Misaki	20	F	0593
		Mizuki	22	F	0559
		Natsuki	13	F	0677
		Zamba	23	M	0543
	KCZ	James	26	M	0499
		Koiko	42[a]	F	0281
		Niini	6	M	0737
		Takashi	31	M	0316
	PRI	Ai	44[a]	F	0434
		Akira	44[a]	M	0435
		Ayumu	19	M	0608
		Chloe	39	F	0441
		Cleo	19	F	0609
		Gon	54[a]	M	0437
		Mari	44[a]	F	0274
		Pal	19	F	0611
		Pan	36	F	0440
		Pendesa	43	F	0095

Note. The “Age” column shows the participant age when they first participated in the experiment. In the “Sex” column, F and M denote female and male, respectively. [a] These ages were estimated. [b] Great Ape Information Network: https://shigen.nig.ac.jp/gain/

Housing

Participant apes at each site were housed in social groups [bonobos at Kumamoto Sanctuary (KS): two groups, six individuals in total; chimpanzees at KS: one group, six individuals in total; chimpanzees at Kyoto City Zoo (KCZ): one group, six individuals in total; chimpanzees at Primate Research Institute (PRI): two groups, eleven individuals in total], and spent most of the day in outdoor or semi-outdoor enclosures that were furnished with hammocks, structures, and other devices to allow apes to freely exercise in three-dimensional complex environments (bonobos at KS: two separate adjacent enclosures, 93.4 m^2 or 95 m^2 × 4.1 m; chimpanzees at KS: three connected enclosures, approximately 300 m^2 in total; chimpanzees at KCZ: two connected areas, 200 m^2 each; chimpanzees at PRI: approximately 700 m^2, every other day) and were also connected to several indoor enclosures. The apes received meals several times per day comprising fresh vegetables, fruits, and other foods depending on the facility (e.g., monkey chow, nutritionally balanced biscuits, etc.). Additional enrichment items were provided depending on the facility (e.g., packages of food pieces, pieces of burlap bags, branches, and leaves, etc.) to facilitate foraging activities or to improve comfort. Water was freely available from taps.

Stimuli and procedure

We cropped the original animation materials to 6 s (originally 9 s) because we expected the apes to lose interest in monotonous stimuli earlier than human infants. To match the size of the figures (visual angle) with those from Morita et al. (2012) as much as possible, we slightly enlarged the stimuli materials, i.e., by 1.465% (originally 874 × 610 pixels), and placed them on a gray background (1920 × 1080 pixels) that was the same color as the background of the original material.

Experiment 1 took place in February and April 2019 for chimpanzees and bonobos at KS, respectively; and in June (and September for one individual) 2019 at KCZ. Experiment 2 took place in May and June 2019 for chimpanzees and bonobos, respectively, and in September 2019 at KCZ. Experiment 3 took place in February 2020 at PRI.

Analysis

The gaze data was first processed with the default Tobii Fixation Filter. Following the previous study (Morita et al. 2012), the sizes of the elbow and face areas of interest (AOIs) were set at approximately 1.5% of the size of the whole stimuli (i.e., 1920 × 1080 pixels). In the human, robot, and biped-ape animations, the position of the whole body on the screen moved according to the elbow movements, unlike in Morita et al. (2012). We set the AOIs so that they tracked the corresponding body parts throughout the animation. To do this, we manually chose keyframes (i.e., onset and offset of movements), placed AOIs on the corresponding body parts in the keyframes, and let Tobii Studio move the AOIs linearly between the keyframes.

Pupillometry Pre-Processing

We pre-processed the pupillometry data using an R package, PupillometryR v.0.0.3 (Forbes 2020), while carefully checking that any processing did not yield a large artifact. The data processing comprising three steps. (1) Averaging: we used the first 900 (KS and PRI) or 180 data points (KCZ) in each trial, which approximately corresponded to 3 s (data were collected at 300 Hz and 60 Hz, respectively). We calculated the average pupil diameter across the left and right eyes (Figs. S3.10, S3.13, S3.16 show the data for the whole trial for illustrative purposes). When either the left or right pupil diameter was not recorded, the data point was regarded missing. Note that we specified the time-windows for the analysis based on the number of recorded data points rather than directly based on the time from stimulus onset. Thus, the time inferred from the number of data point may not be exactly the same as the time from stimulus onset. For example, we regarded the 900th data points collected at 300 Hz may not exactly correspond to 3 s from the stimulus onset). (2) Time-bin: we averaged data in 10- (KS and PRI) or 2-point time-bins (KCZ) to reduce the dataset size and accommodate differences in the amount of data points. When at least one data point was missing within a time-bin, the time-bin was regarded missing. We then removed trials with > 15 % missing values (Figs. S3.11, S3.14, S3.17). (3) Baseline correction: the baseline value was defined as the average of the first 9−14 time-bin values, which corresponded to approximately 300−500 ms from the animation onset. When some time-bin values were missing in the first 9−14 time-bins, baseline value was calculated while excluding those missing values. We defined this baseline, rather than positioning the baseline around the exact onset of animation, to avoid obtaining a spurious post-baseline correction difference between conditions caused by variations in pupil diameter at the animation onset. We baseline-corrected the following time-bin values using that baseline, and then averaged the 61−90th values, which approximately corresponded to 2−3 s from the animation onset (Figs. S3.12, S3.15, S3.18). Again, average was taken while excluding missing values. The averaged values were subsequently analyzed using paired *t* tests.

Confidence intervals for Cliff's d

Given the estimated effect (d), its variance (s_d^2), and the critical value of the normal distribution at the level of $\alpha = 0.05$ ($z_{\alpha/2}$), confidence intervals (CIs) were calculated as follows (Feng and Cliff 1995, as cited in Long et al. 2003):

$$CI_{upper/lower} = \frac{d - d^3 \pm z_{\alpha/2} \times \sqrt{s_d^2} \times \sqrt{(1 - d^2)^2 + {z_{\alpha/2}}^2 \times s_d^2}}{1 - d^2 + {z_{\alpha/2}}^2 \times s_d^2}$$

Supplementary file 2. A chimpanzee (shown at the left top corner) viewing the animation of a human engaging in possible movements in experiment 1. Fixation points are shown as red dots.

Supplementary file 3. A chimpanzee (shown at the left top corner) viewing the animation of a human engaging in impossible movements in experiment 1. Fixation points are shown as red dots.

Supplementary file 4. A chimpanzee (shown at the left top corner) viewing the animation of a robot engaging in possible movements in experiment 1. Fixation points are shown as red dots.

Supplementary file 5. A chimpanzee (shown at the left top corner) viewing the animation of a robot engaging in impossible movements in experiment 1. Fixation points are shown as red dots.

Supplementary file 6. A bonobo (shown at the left top corner) viewing the animation of an ape (standing bipedally) engaging in possible movements in experiment 2. Fixation points are shown as red dots. The sounds included in the video were not played for the bonobos.

Supplementary file 7. A bonobo (shown at the left top corner) viewing the animation of an ape (standing bipedally) engaging in impossible movements in experiment 2. Fixation points are shown as red dots. The sounds included in the video were not played for the bonobos.

Supplementary file 8. A chimpanzee (shown at the left top corner) viewing the animation of an ape (standing quadrupedally) engaging in possible movements in experiment 3. Fixation points are shown as red dots.

Supplementary file 9. A chimpanzee (shown at the left top corner) viewing the animation of an ape (standing quadrupedally) engaging in impossible movements in experiment 3. Fixation points are shown as red dots.

Supplementary Results

Time spent looking at the screen

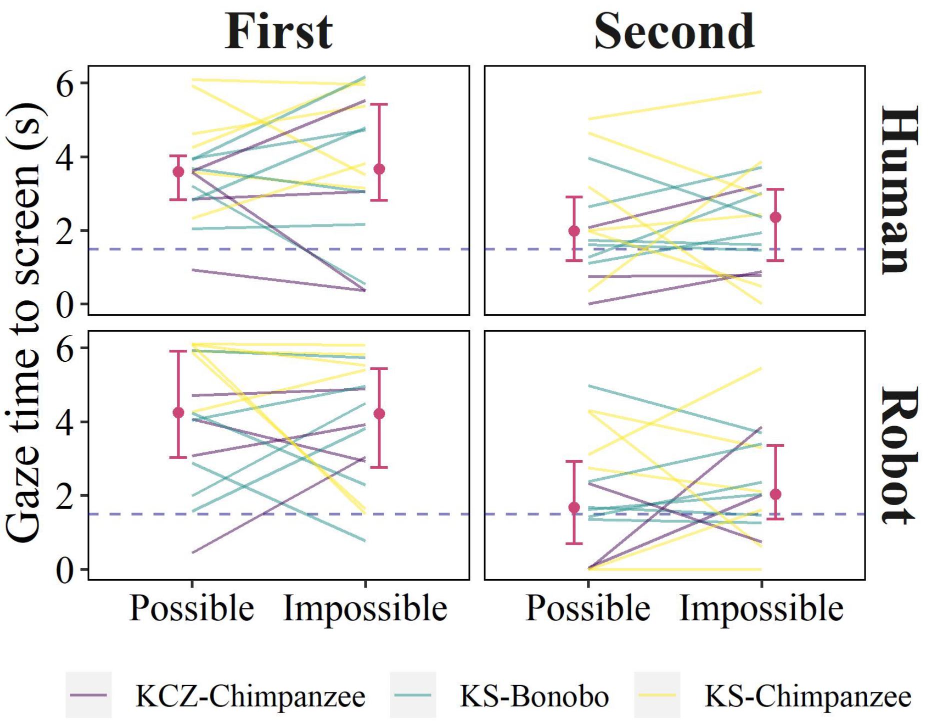

Fig. S3.1 Time spent viewing the screen in experiment 1. The dots and error bars show the median and the first/third quantiles, respectively. The dashed horizontal line shows the value of 1.5 (s), which was used as an inclusion criterion.

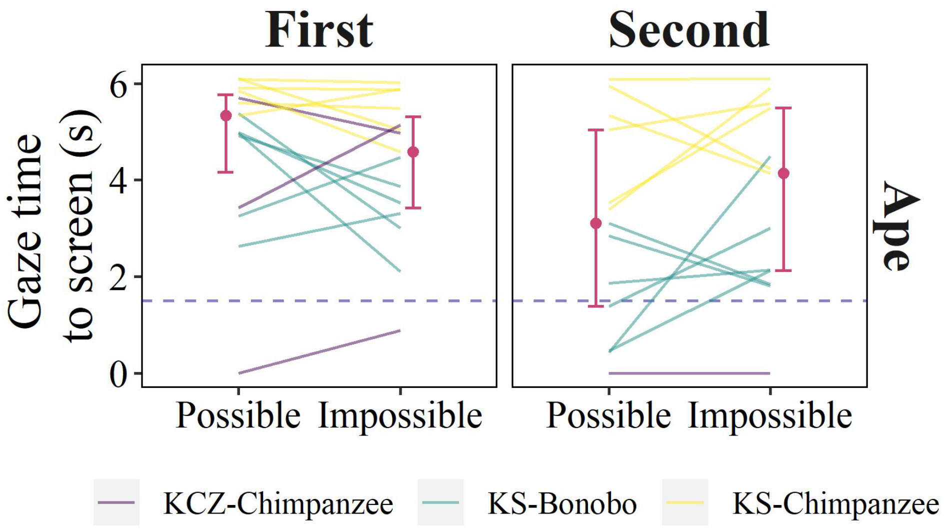

Fig. S3.2 Time spent viewing the screen in experiment 2. The dots and error bars show the median and the first/third quantiles, respectively. The dashed horizontal line shows the value of 1.5 (s), which was used as an inclusion criterion.

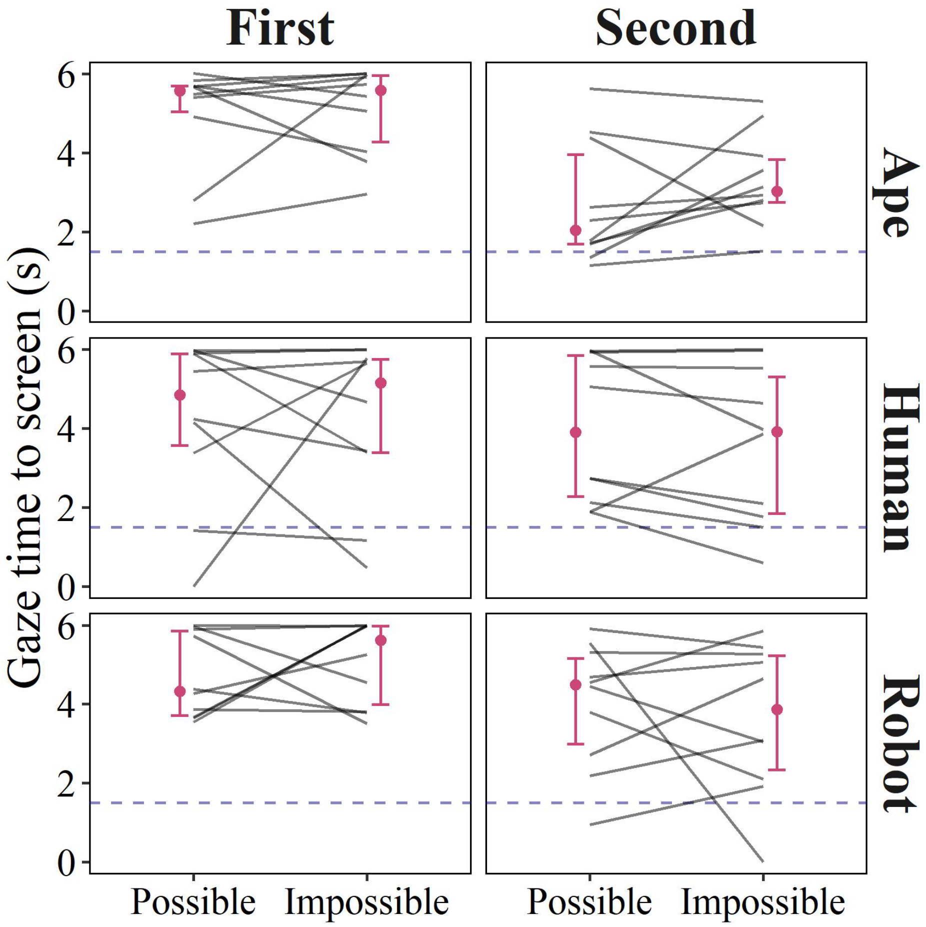

Fig. S3.3 Time spent viewing the screen in experiment 3. The dots and error bars show the median and the first/third quantiles, respectively. The dashed horizontal line shows the value of 1.5 (s), which was used as an inclusion criterion.

Proportion of time spent looking at the elbows in each trial

Table S3.2 Overview of Wilcoxon signed-rank test results for each trial. Trials in which the apes did not view the screen for ≥ 1.5 s were excluded.

Experiment	Model	Trial	*n*	*V*	*p*
Experiment 1	Human	1st	13	62	0.077
		2nd	7	23	0.156
	Robot	1st	13	45	0.677
		2nd	6	3	0.156
Experiment 2	Ape (bipedally-standing)	1st	14	47	0.946
		2nd	9	6	0.055
Experiment 3	Ape (quadrupedally-standing)	1st	10	9	0.844
		2nd	8	14	1
	Human	1st	7	23	0.156
		2nd	9	28	0.570
	Robot	1st	10	52	0.010
		2nd	8	12	0.461

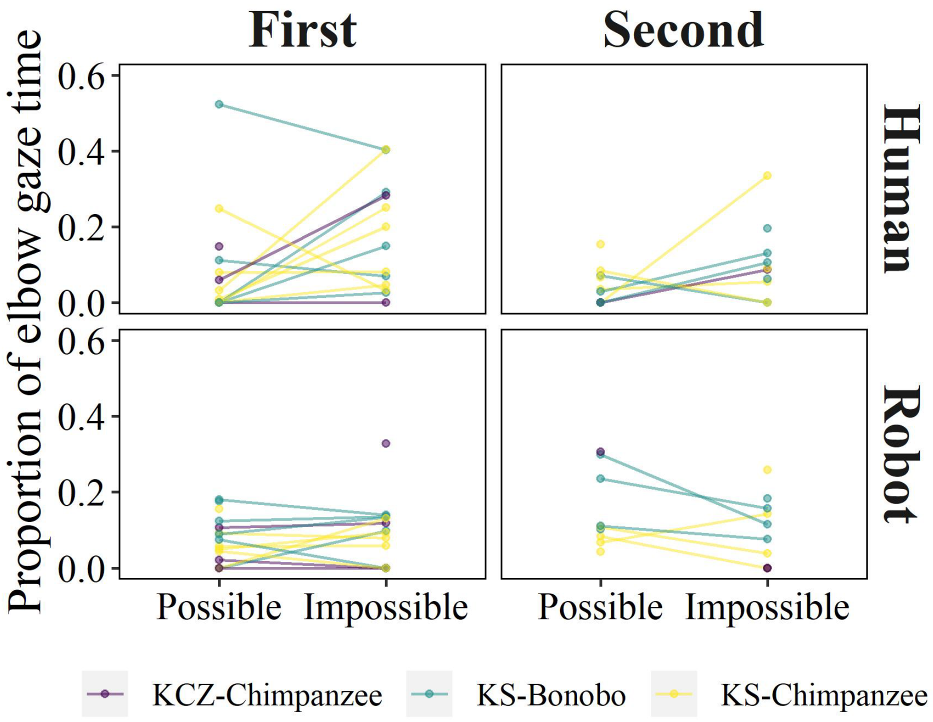

Fig. S3.4 Proportions of time spent looking at elbows when viewing human and robot animations (experiment 1). Data from some individuals who lacked data from either condition (possible or impossible) were included for the purpose of visualization, but were not included in the statistical analysis.

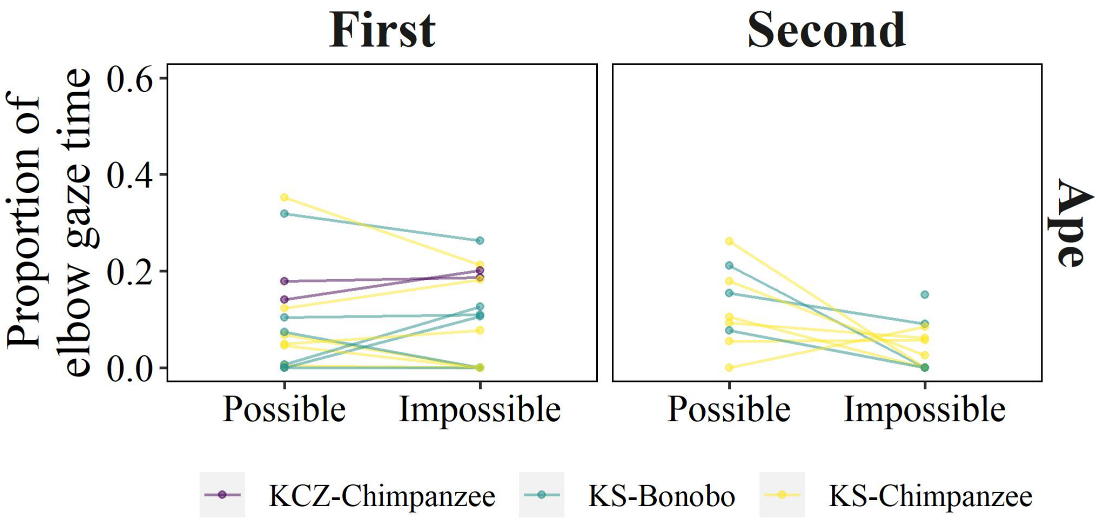

Fig. S3.5 Proportions of time spent looking at elbows when viewing nonhuman ape animations (experiment 2). Data from some individuals who lacked data from either condition (possible or impossible) were included for the purpose of visualization, but were not included in the statistical analysis.

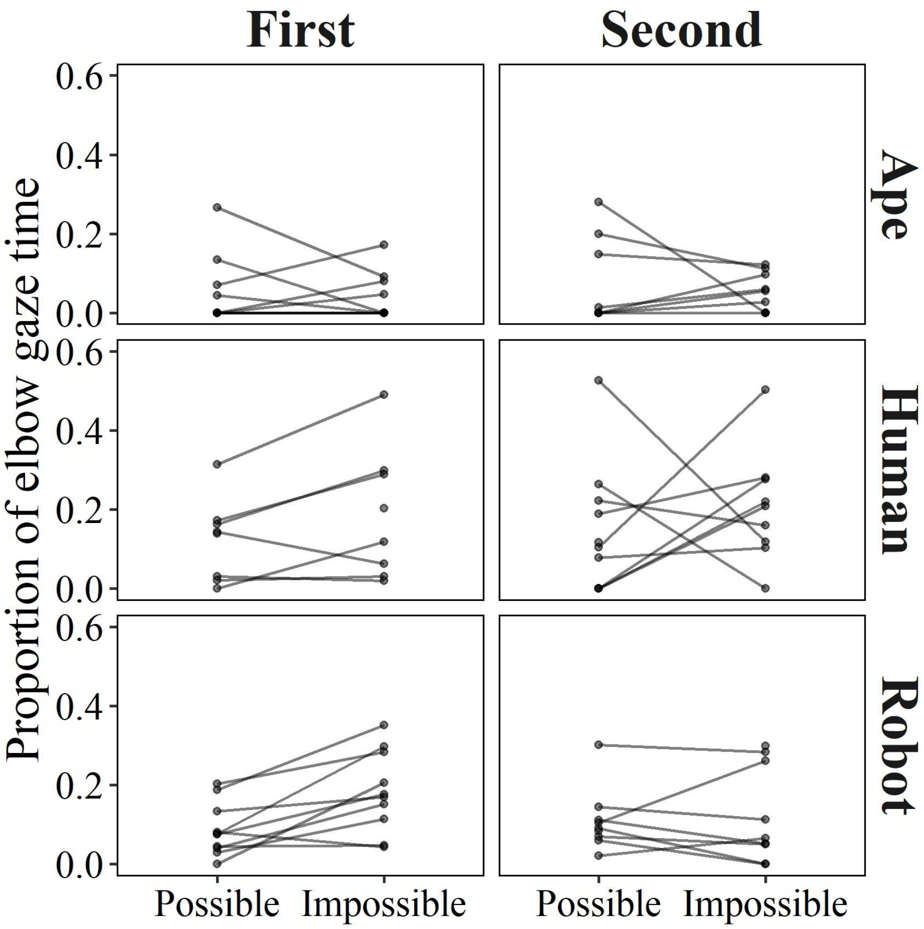

Fig. S3.6 Proportions of time spent looking at elbows when viewing nonhuman ape animations (experiment 3). Data from some individuals who lacked data from either condition (possible or impossible) were included for the purpose of visualization, but were not included in the statistical analysis.

Pupillometry

Experiment 1

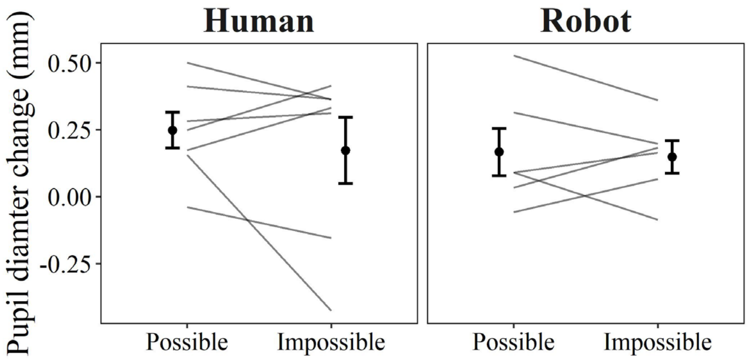

Fig. S3.7 Change in pupil diameter in experiment 1. Dots and error bars show the mean and SEM.

Experiment 2

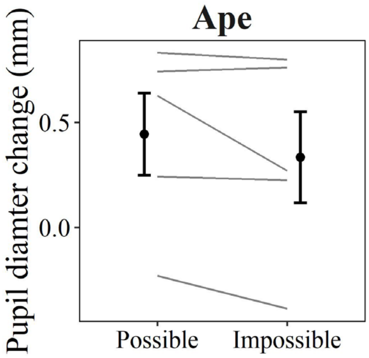

Fig. S3.8 Change in pupil diameter in experiment 2. Dots and error bars show the mean and SEM.

Experiment 3

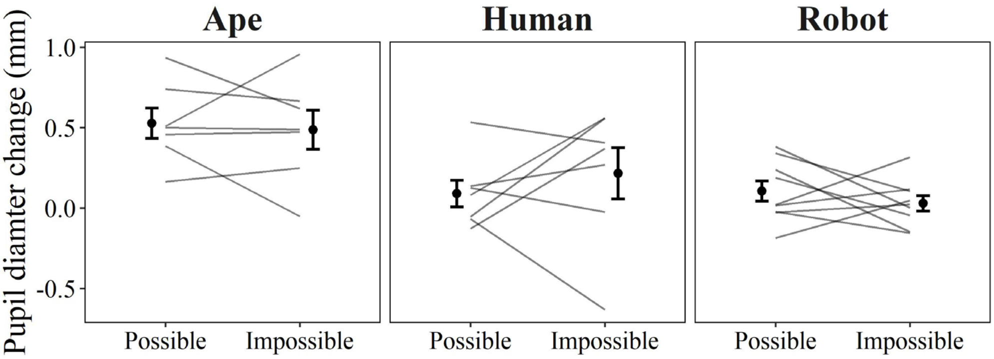

Fig. S3.9 Change in pupil diameter in experiment 3. Dots and error bars show the mean and SEM.

Pupillometry time-series visualization

Experiment 1

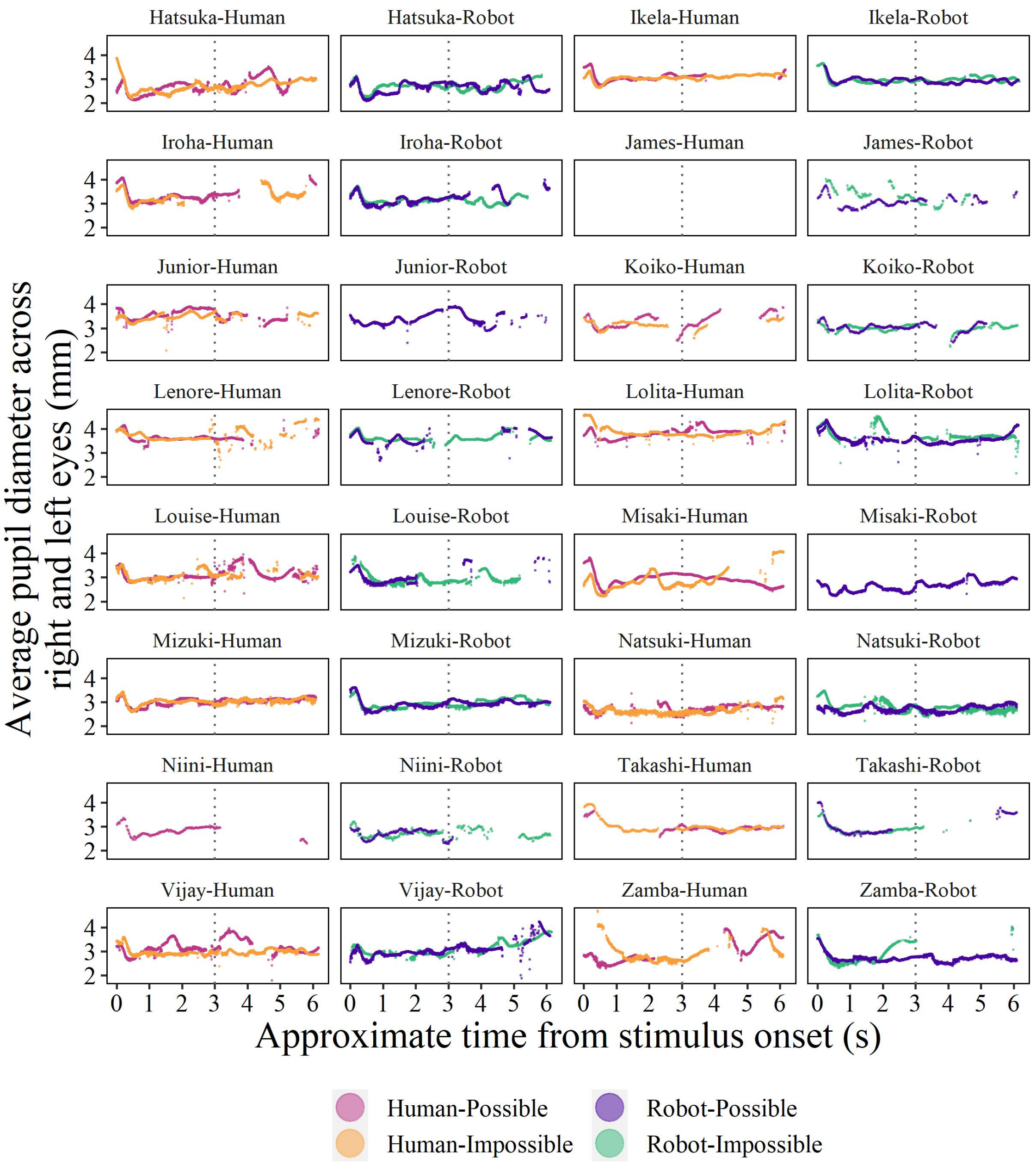

Fig. S3.10 Pupil diameter, averaged across the right and left eyes in experiment 1. One blank panel (James-Human) was inserted to increase the ease of viewing. The first 900 (KS) or 180 (KCZ) data points (to the left of the vertical dotted line) were used in the sebsequent step.

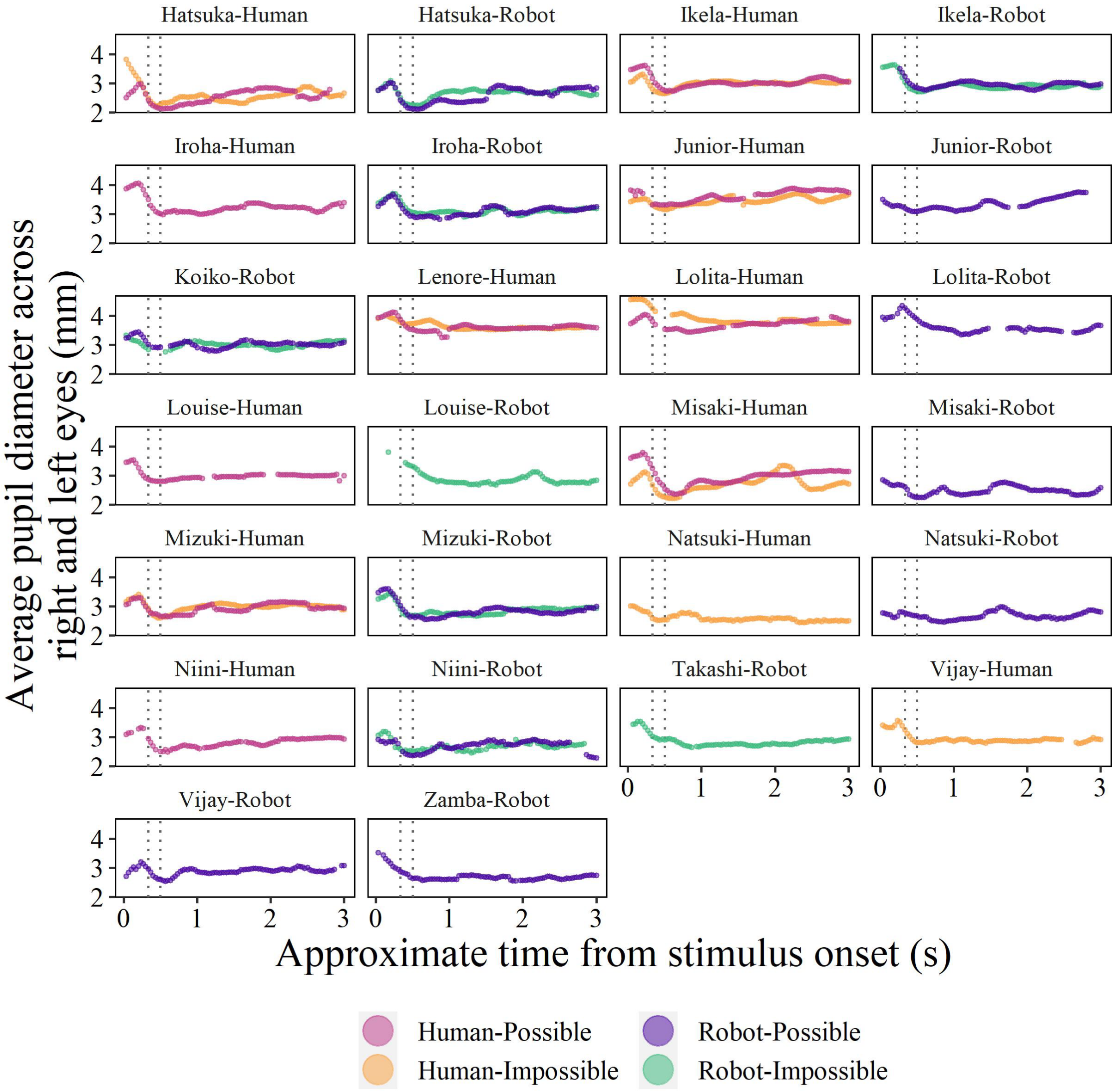

Fig. S3.11 Pupil diameter, averaged into time-bins in experiment 1. The average of the first 9−14 time-bin values (the areas between the vertical dotted lines) was used as the baseline in the subsequent step.

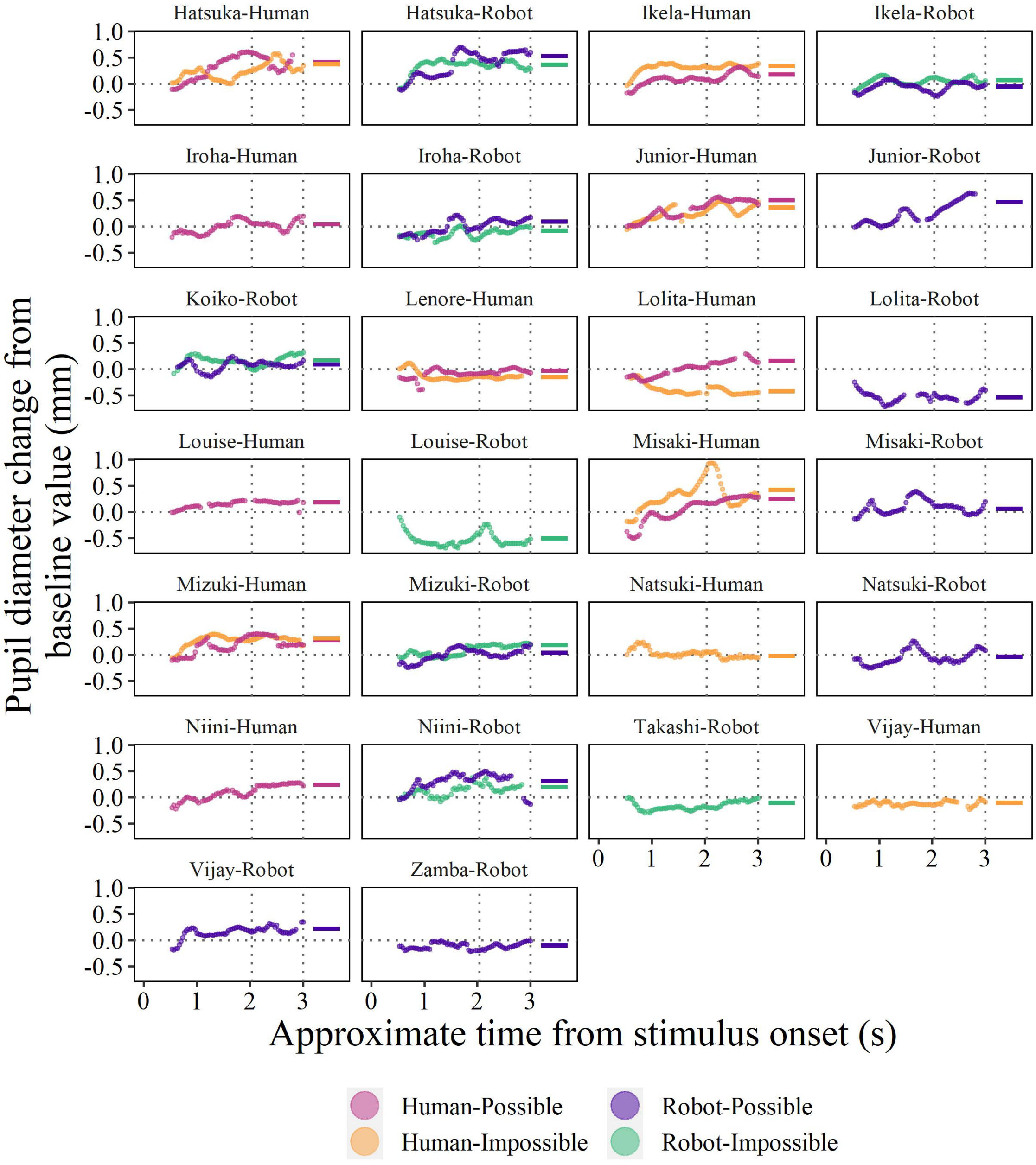

Fig. S3.12 Change in pupil diameter from the baseline in experiment 1. The 61−90 values (the areas between the vertical dotted lines) were averaged and subsequently compared across conditions. The averaged values are indicated by the short horizontal lines at the right side of each panel.

Experiment 2

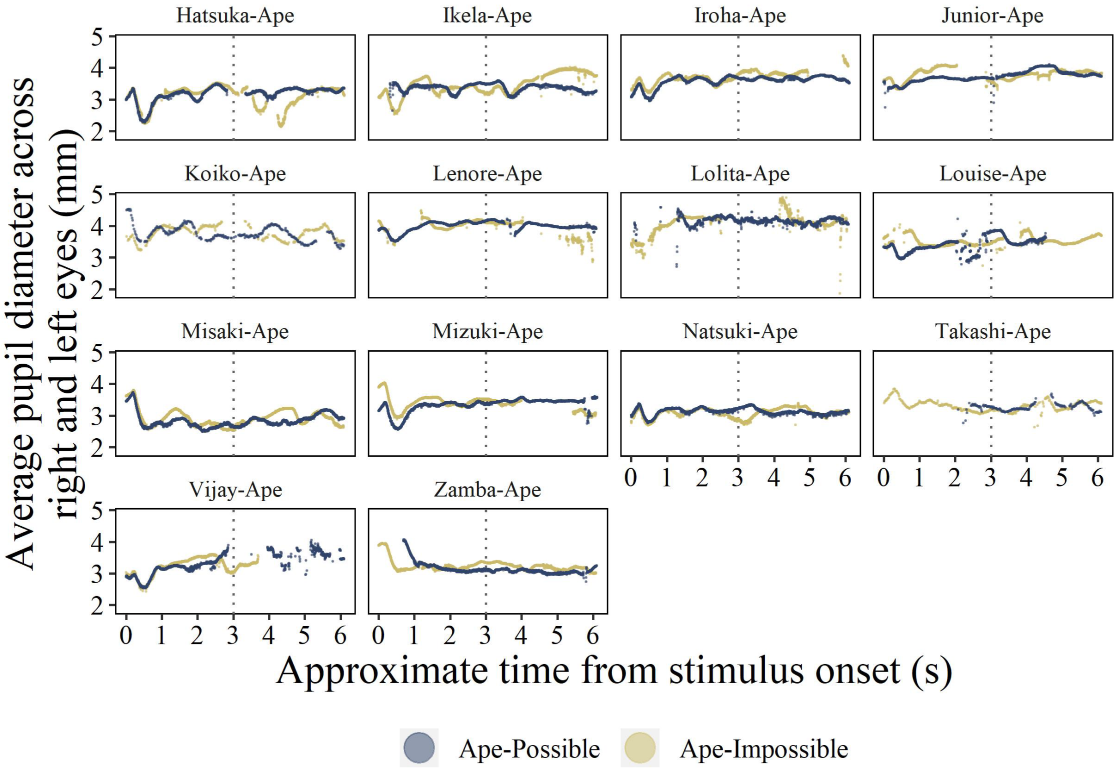

Fig. S3.13 Pupil diameter, averaged across the right and left eyes in experiment 2. The first 900 (KS) or 180 (KCZ) data points (to the left of the vertical dotted line) were used in the sebsequent step.

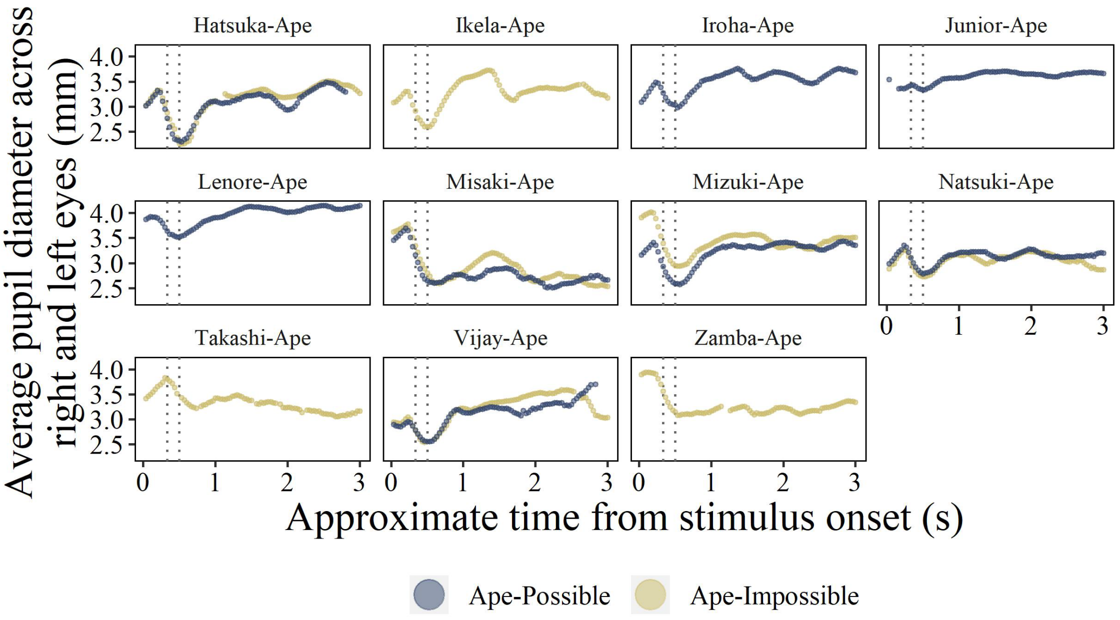

Fig. S3.14 Pupil diameter, averaged into time-bins in experiment 2. The average of the first 9−14 time-bin values (the areas between the vertical dotted lines) was used as the baseline in the subsequent step.

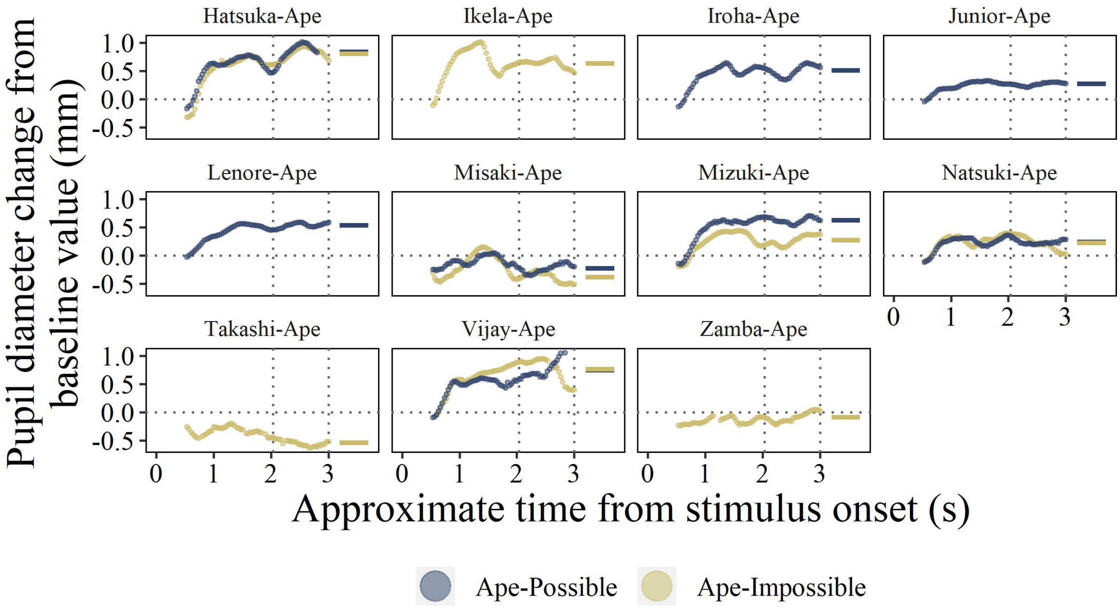

Fig. S3.15 Change in pupil diameter from the baseline in experiment 2. The 61−90 values (the areas between the vertical dotted lines) were averaged and subsequently compared across conditions. The averaged values are indicated by the short horizontal lines at the right side of each panel.

Experiment 3

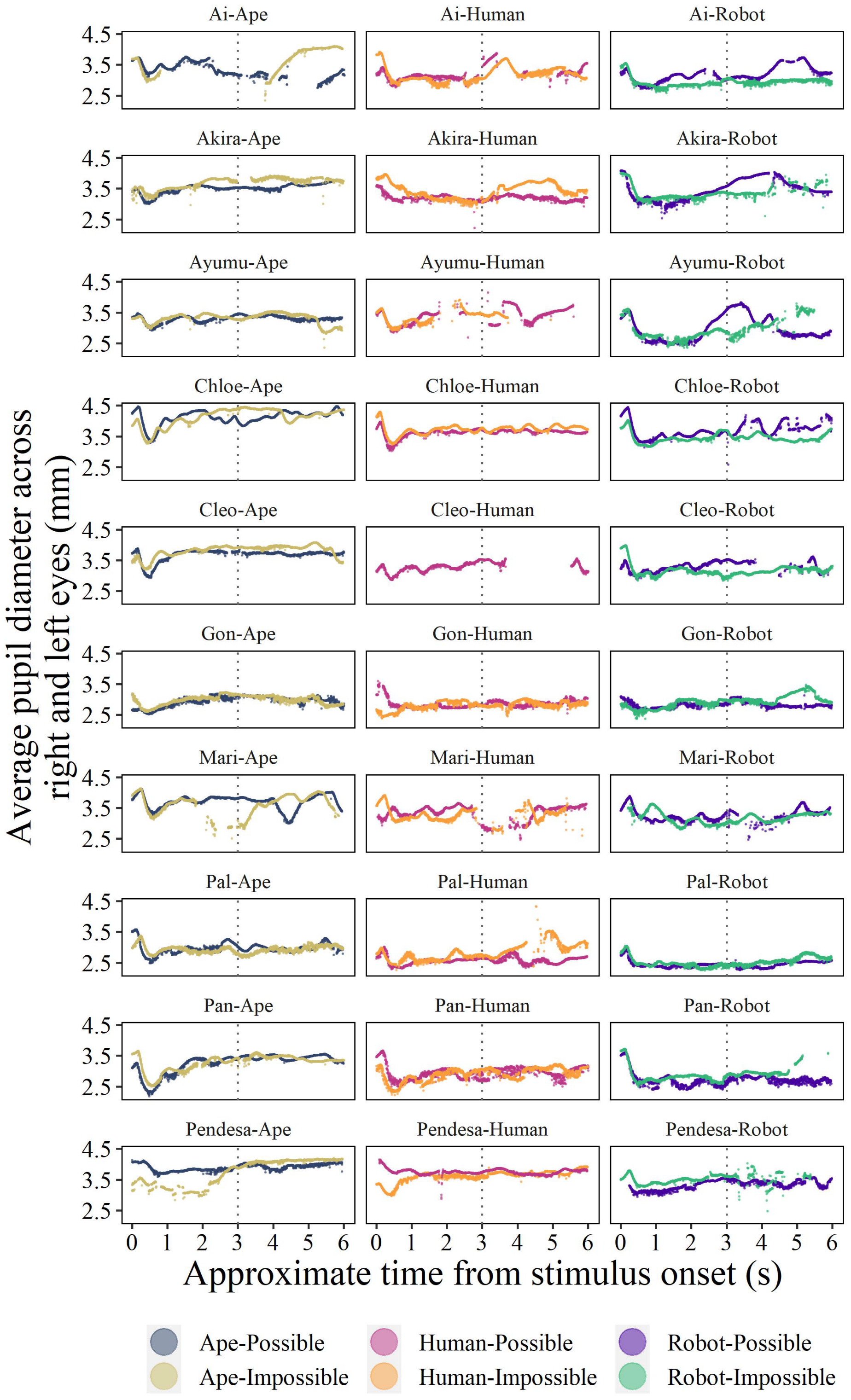

Fig. S3.16 Pupil diameter, averaged across the right and left eyes in experiment 3. The first 900 data points (to the left of the vertical dotted line) were used in the sebsequent step.

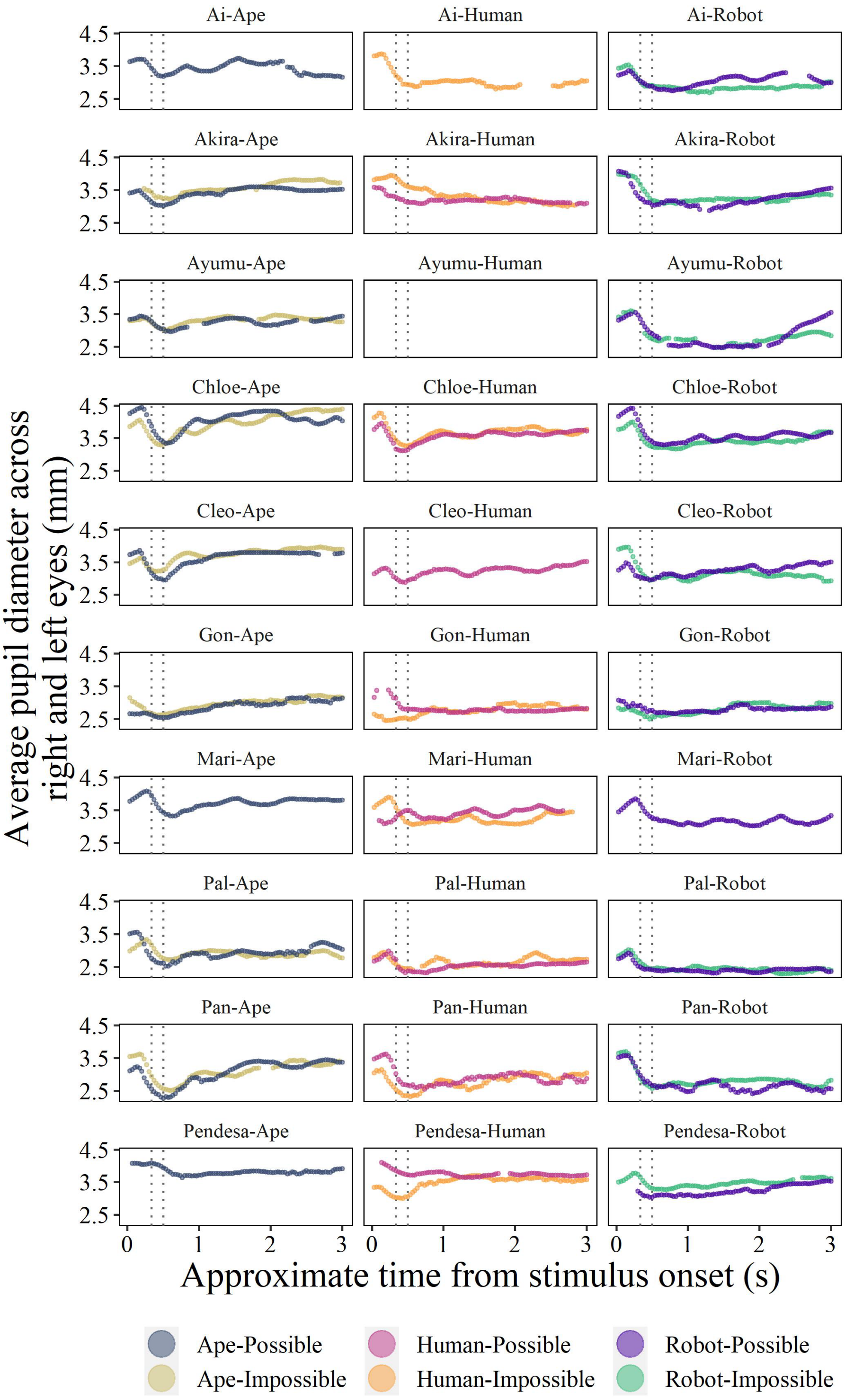

Fig. S3.17 Pupil diameter, averaged into time-bins in experiment 3. One blank panel (Ayumu-Human) was inserted to increase the ease of viewing. The average of the first 9−14 time-bin values (the areas between the vertical dotted lines) was used as the baseline in the subsequent step.

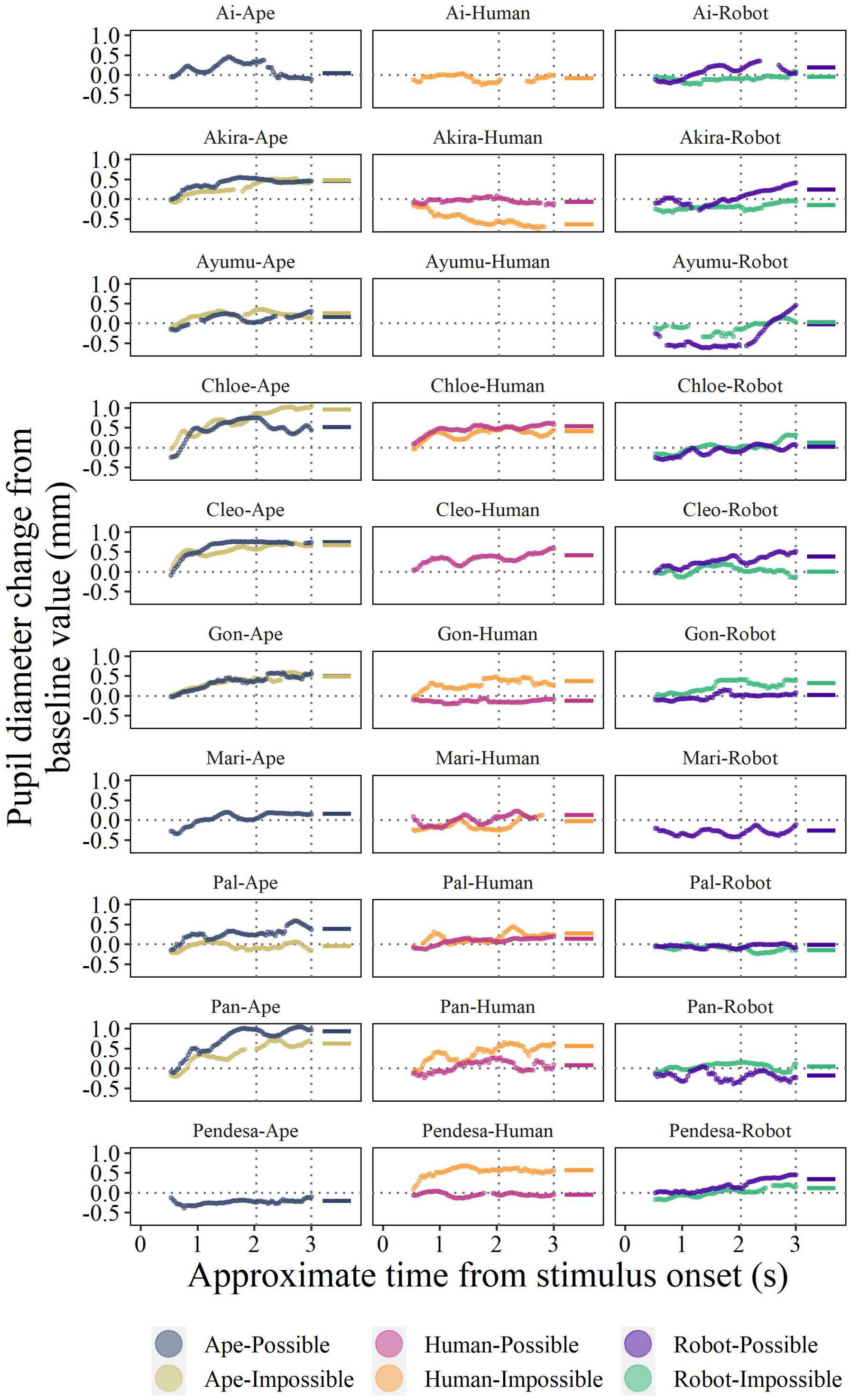

Fig. S3.18 Change in pupil diameter from the baseline in experiment 3. One blank panel (Ayumu-Human) was inserted to increase the ease of viewing. The 61−90 values (the areas between the vertical dotted lines) were averaged and subsequently compared across conditions. The averaged values are indicated by the short horizontal lines at the right side of each panel.

Supplementary References

Feng D, Cliff N (1995) Comparisons of power and size of *d* and *t* statistics under normal and nonnormal assumptions. University of Southern California, Los Angeles (Unpublished manuscript).

Forbes SH (2020) PupillometryR: an R package for preparing and analysing pupillometry data. J Open Source Software 5:2285. https://doi.org/10.21105/joss.02285

Long JD, Feng D, Cliff N (2003) Ordinal analysis of behavioral data. In: Schinka JA, Velicer WF (eds) Handbook of psychology: research methods in psychology, vol 2. Wiley, Hoboken, NJ, pp 635–661. https://doi.org/10.1002/0471264385.wei0225

Morita T, Slaughter V, Katayama N et al (2012) Infant and adult perceptions of possible and impossible body movements: an eye-tracking study. J Exp Child Psychol 113:401–414. https://doi.org/10.1016/j.jecp.2012.07.003

Chapter 4

Chimpanzees (*Pan troglodytes*) Exhibit Gaze Bias for Snakes upon Hearing Alarm Calls

4.1 Abstract

Calls of several species of nonhuman animals are considered to be functionally referential. However, the cognitive mechanisms underlying audience behaviors remain unclear. This study used an audiovisual cross-modal preferential-looking paradigm to examine whether captive chimpanzees spontaneously associated a conspecific call with images of a corresponding item. Chimpanzees were presented with videos of snakes and fruit side-by-side while hearing playbacks of alarm calls, food-associated calls, or no sound (as a baseline condition). Chimpanzees looked at videos of snakes for longer when hearing alarm calls compared with food calls or baseline. However, chimpanzees did not look at videos of fruit for longer when hearing food calls compared with baseline. An additional experiment tested whether chimpanzees' gaze bias to the snake videos was driven by negative affective states in general via affect-driven attention biases. When chimpanzees were presented with the same snake and fruit videos while hearing playbacks of conspecific screams or no sound, they exhibited no gaze bias for snake videos. These results suggest that chimpanzees spontaneously associated alarm calls with images of a potential threat in a preferential-looking experiment and that this response was not simply driven by an affective state matching process. These findings should be interpreted in consideration of a procedural limitation related to pseudoreplication in the experimental stimuli.

Keywords: Chimpanzee; Cross-modal preferential looking; Vocalization; Alarm call; Snake

4.2 Introduction

Previous studies have suggested that some calls of nonhuman animals are referential, at least functionally, in that audience animals respond as if the vocalizations indicate specific external events or objects (reviewed in Macedonia and Evans 1993; Seyfarth and Cheney 2003; but see also Wheeler and Fischer 2012, for a critical view). For example, in several species of birds and mammals, distinct alarm calls in response to the presence of predators have been observed to induce audience animals to exhibit adaptive evasion responses according to the nature of predator movements (reviewed in Suzuki 2016; Townsend and Manser 2013). Moreover, carefully designed playback experiments suggest that audience animals are not simply responding to calls in a fixed manner (Clay and Zuberbühler 2011; Evans and Evans 2007; Seyfarth and Cheney 1990; Slocombe and Zuberbühler 2005; Suzuki 2018, 2020; Zuberbühler 2000; Zuberbühleret al. 1999). For example, in a field experiment (Seyfarth and Cheney 1990), vervet monkeys (*Cercopithecus aethiops*) repeatedly heard playbacks of either conspecific alarm calls or alarm calls of a sympatric bird species (superb starlings: *Spreo superbus*). Monkeys progressively reduced their responses to playback (i.e., looking toward the speaker), which indicates that they became habituated to it. Monkeys were found to transfer this habituation between playbacks of conspecific calls elicited by eagles and bird alarm calls elicited by raptors as if the two types of call redundantly informed monkeys of similar threats. Furthermore, an experiment with fowls (*Gallus gallus*) reported that individuals exhibited greater search effort for food when they heard food calls compared with when they heard alarm calls but did not differentiate search effort when they had recently discovered food by themselves (Evans and Evans 2007). A previous study (Slocombe and Zuberbühler 2005) suggested that chimpanzee (*Pan troglodytes*) food grunts functioned referentially in an audience chimpanzee in an experimentally induced situation. The chimpanzees first learned that the two locations were baited with cardboard tubes containing either pieces of apple or bread. Subsequently, when the chimpanzees heard a series of food grunts emitted by groupmates in response to apples or bread, the individual exhibited greater search effort for the corresponding food item (for a similar finding with bonobos [*Pan paniscus*], see Clay and Zuberbühler 2011). These results indicate that audience animals do not just exhibit an adaptive response to each call in a fixed manner but adjust their responses more flexibly according to the types of conspecific calls and the contexts in which they heard those calls.

This body of evidence suggests that animals may recollect representations of relevant items upon hearing the calls (Evans and Evans 2007; Seyfarth and Cheney 1990; Zuberbühler et al. 1999). Another line of evidence supporting this claim comes from cross-modal matching experiments, in which participants are sequentially presented with auditory and visual stimuli, and observed for spontaneous responses to the visual referents of the auditory stimuli (Suzuki 2016). In studies of wild tits, birds approached a wooden stick that simulated snake-like movements only when they heard snake-specific alarm calls of conspecifics (Suzuki 2018; or heterospecifics: Suzuki 2020) but not when they heard an irrelevant call, or when the stick exhibited irrelevant movement (Japanese great tits [*Parus minor*]: Suzuki 2018; coal tits [*Periparus ater*]: Suzuki 2020). Thus, tits appeared to be primed to detect snakes when they heard snake-specific calls. In a somewhat related study (Shibasaki et al. 2014), captive Japanese macaques (*Macaca fuscata*) were presented with images of a snake and a flower side-by-side while simultaneously hearing playbacks of either alarm calls or irrelevant contact calls. Monkeys looked at the snake for longer than the flower when hearing the alarm call, although this effect was limited to when the snakes were presented on the left side of the screen. Similarly, human (*Homo sapiens*) infants looked at videos of snakes for longer when hearing frightened human voices than when hearing happy voices (DeLoache and LoBue 2009).

The current study aimed to contribute to this accumulating evidence by testing whether chimpanzees spontaneously associated conspecific calls with specific visual referents in cross-modal preferential-looking experiments following a design implemented in a previous study of monkeys (Shibasaki et al. 2014) with several modifications, as described in the following text. Chimpanzees are known to flexibly adjust their call utterances and behavioral responses to conspecific calls depending on context (e.g., Kalan et al. 2015; Schel et al. 2013; Slocombe and Zuberbühler 2005, 2006). For example, chimpanzees have been reported to adjust utterances of alarm calls in response to a realistic model of a snake, possibly according to the knowledge status of the audience chimpanzees (i.e., whether audience members had already seen the model snake and whether audience members had heard alarm calls: Crockford et al. 2012; for a similar trend, see also Girard-Buttoz et al. 2020), or on the basis of whether audience presence was simulated by playbacks of alarm calls, indicative of their awareness of threat, or playbacks of an irrelevant call, indicative of their unawareness of threat (Crockford et al. 2017). Moreover, chimpanzees were more likely to exhibit marking-like behaviors (i.e., repositioning of their body and gaze alteration between audience and snake) when audiences had not seen the snake compared with when audiences had seen the snake (Crockford et al. 2017). Alarm calls in combination with marking behaviors more effectively induced audiences to cautiously approach the snake compared with alarm calls alone.

Although the relationships between vocalizations and audience behavior have been researched in some monkey species, it remains unclear whether and to what extent chimpanzees associate calls with relevant objects. Chimpanzees have been reported to emit alarm calls such as alarm barks, screams, and “hoos” in response to various types of threats such as snakes and larger-bodied mammals including leopards and buffalos (Crockford and Boesch 2003; Goodall 1986, Chapter 6). In playback experiments (Crockford et al. 2015, 2017), chimpanzees were found to pay more attention to a hidden speaker when hearing playbacks of alarm hoo calls compared with another hoo call emitted in a resting context. However, it is unclear whether and to what extent those calls convey properties of a specific event to the audience (Crockford et al. 2015; Dezecache and Berthet 2018; Dezecache et al. 2019). Regarding food-associated calls, previous studies suggest that call production is facilitated by larger food resources (Brosnan and de Waal 2000; Hauser et al. 1993 [divisible food resources in particular]; Hauser and Wrangham 1987; Leroux et al. 2021 [combinatorial use of calls in particular]; Slocombe et al. 2010; but see O'Bryan 2015 for a null result) and more preferable types of food (Kalan and Boesch 2015; Fedurek and Slocombe 2013; but see O'Bryan 2015 for an opposite result). Food-associated calls have been reported to attract other chimpanzees to a food patch, at least in some cases (Kalan and Boesch 2015; O'Bryan et al. 2021; but see O'Bryan 2015 for a null result). In particular, chimpanzee food grunts have been suggested to exhibit different acoustic profiles depending on the characteristics of food, such as the relative value/preference or size of the food (Hallberg et al. 2003; Kalan et al. 2015 [but only for one tree species]; Slocombe and Zuberbühler 2005, 2006 [in captivity but not in a wild habitat]; see also O'Bryan 2015 for a null result). Preliminary evidence suggests that audience chimpanzees may use such acoustic differences to guide subsequent behaviors (Kalan et al. 2015; Slocombe and Zuberbühler 2005). Similarly, when captive chimpanzees were presented with four images of different food items on a touchscreen and heard a playback of food barks, chimpanzees were reported to spontaneously select an image depicting a food item of similar value as the one that elicited the food bark more often than expected by chance (Hallberg et al. 2003; Gibbons 2007). However, this line of inquiry has yielded mixed results (O'Bryan 2015), and the cognitive underpinnings of audience behavioral responses to those calls remain unclear. In the current study, we sought to examine whether chimpanzees spontaneously match either alarm calls or food grunts to relevant images of snakes and fruit, respectively.

4.3 Experiment 1

Chimpanzees were presented with videos of snakes and fruit side-by-side while hearing playbacks of either alarm calls or food grunts. We measured chimpanzees' gaze to each type of video using an eye tracker. If chimpanzees had associations between alarm calls and snakes or between food calls and fruits, they would be expected to look at snake videos for longer when hearing alarm calls than when hearing food grunts and to look at fruit videos for longer when hearing food grunts than when hearing alarm calls.

4.3.1 Methods

Participants

Six chimpanzees at Kumamoto Sanctuary (KS), Wildlife Research Center, Kyoto University, participated in experiment 1 (five females and one male, 10−24 years old; Table 4.1). Chimpanzees lived in a group comprising these six individuals. Chimpanzees spent the daytime in open-air outdoor enclosures (in total 294 m^2) with occasional access to indoor enclosures, in which they received meals and slept at night. These enclosures were equipped with complex structures such as climbing towers, platforms, hammocks, ropes, and/or small trees to allow chimpanzees to move and climb freely. Chimpanzees received meals comprising fresh vegetables, fruits and monkey chow three times a day, and, in between, small amounts of nuts and seeds in small packages or juice in tubes for enrichment. They had free access to water from the taps located in both outdoor and indoor enclosures. Chimpanzees were not deprived of food or water for the studies. The study depended on their voluntary participation. Animal husbandry and study protocols complied with the Guide for Animal Research Ethics provided by the Wildlife Research Center, Kyoto University (WRC-2018-KS006A).

Stimuli

Visual stimuli were video clips of snakes and fruit (400 px × 400 px) presented on a black screen (1280 px × 720 px) side-by-side. Each video clip of snakes or fruit comprised four 3-s clips depicting different items in each category (12 s in total; Fig. 4.1a), which were presented in the same order across trials and participants. Snake videos depicted four species of snakes found in Japan: the Japanese rat snake (*Elaphe climacophora*), Japanese striped snake (*Elaphe quadrivirgata*), Japanese pit viper (*Gloydius blomhoffii*), and tiger keelback (*Rhabdophis tigrinus*). The chimpanzees in this study were likely to have observed one or more of these species because they are commonly found outdoors in Japan, and the chimpanzees have explored their outdoor compounds freely since their youth (chimpanzees in experiment 1: Idani and Hirata 2006; chimpanzees in experiment 3: Matsuzawa 2006). Snakes exhibited sinusoidal motions in each of the 3-s snake video clips. Fruit videos depicted pieces of fruit (apple, pineapple, banana, or persimmon) on a silver plate. Chimpanzees were often fed pieces of fruit on this plate for lunch. In the video, a human experimenter picked up and held one piece of fruit. This action was intended to roughly control for the difference in the amount of movement between the fruit and snake videos.

Auditory stimuli were alarm calls (probably those described as “wraaa”: Goodall 1986, Chapter 6; Marler and Tenaza 1977; or “Waa Barks”: Schel et al. 2013) and food grunts (described as “rough grunts”: Marler and Tenaza 1977; Slocombe and Zuberbühler 2006; or “food grunts”: Goodall 1986, Chapter 6). For each call type, we used two calls emitted by two of the female chimpanzee participants (Fig. 4.1b). The calls were recorded using a video camera (HDR-CX430V, Sony Marketing Inc., Tokyo, Japan). We recorded alarm calls (0.7 s and 1.0 s of audio were clipped from video recordings and are shown as spectrograms in the left column of Fig. 4.1b) during an incident in which chimpanzees repeatedly emitted alarm calls, and an experienced caretaker suggested that the chimpanzees were likely to have found a snake in an outdoor enclosure, although we did not observe the snake directly during the incident. The 0.7-s and 1.0-s alarm calls were played eleven and nine times, respectively. The calls were distributed over the entire duration of the 12-s video at arbitrary intervals (Video S4.1 in Supplementary Materials). The same composition was used across trials. We recorded food calls (1.5 s and 3.0 s of audio were clipped from video recordings and are shown as spectrograms in the middle column of Fig. 4.1b) when chimpanzees grunted during feeding times in an experimental booth, during which they could receive pieces of fruit. The 1.5-s and 3.0-s calls were played four and three times, respectively. The calls were distributed over the entire duration of the 12-s video at arbitrary intervals (Video S4.2 in Supplementary Materials). The same composition was used across trials. The playback volumes of the calls were not equated strictly between call types but were adjusted so that the auditory stimuli could be heard clearly inside the experimental booth (although the exact volume level was not recorded at the time, it was estimated to be approximately 70 dBA by post hoc measurement). We used Adobe Audition CC 2017 to create auditory sequences, Adobe After Effects 2019 to combine the visual and auditory stimuli, and Xmedia Recode to reduce the file sizes.

Apparatus

Chimpanzees were invited into an indoor experimental booth (3 m × 3 m × 2 m), either individually or in a dyad for two mother-offspring pairs. Chimpanzees sat inside the experimental booth and viewed the stimuli through a transparent 1-cm-thick polycarbonate panel (Fig. 4.2). The experimental equipment was installed outside the booth. Chimpanzees were eye-tracked with an infrared eye tracker (Tobii TX300, Tobii Technology AB, Stockholm, Sweden) while being presented with visual stimuli on a 23-in. LCD monitor at a viewing distance of approximately 70 cm. The sounds were played through speakers located below the monitor. Before conducting daily experiments, we sprayed antifog liquid on the transparent panel to prevent chimpanzees' breath causing condensation on the panel, which can cause noise in eye movement recording. To reduce chimpanzees' head movements, we allowed chimpanzees to sip juice that was slowly dispensed via a nozzle attached to the transparent panel. Another experimenter also held some of the participants' heads gently (for Hatsuka, Misaki, and Mizuki) to improve head stabilization during the recording. Chimpanzees' eye movements were never explicitly trained in the present study, in accord with previous studies carried out in this sanctuary (overviewed in Hopper et al. 2020).

Procedures

On the first testing day and whenever necessary, we conducted two-point automated calibration, in which small video clips were presented at each reference point. We used the same calibration record for the same participant across trials (days) in each experiment but manually checked the calibration accuracy before each trial. Specifically, we presented nine small icons on the screen, then guided chimpanzees' gaze onto one of the icons and observed whether the recorded gaze deviated from the icon.

At the start of each trial, we presented a small 3-s video clip in which slideshows of chimpanzees were presented with brown-noise (although the exact volume level was not recorded at the time, it was estimated to be approximately 60 dBA by post hoc measurement) to allow chimpanzees to become accustomed to sounds and thereby prevent them from being frightened during the playback of test sounds. A white fixation cross was then presented on a black screen. The test video was played with a key press when the experimenter visually confirmed that chimpanzees appeared to be attending to the screen.

Chimpanzees performed one trial (i.e., 12-s audiovisual stimulus) per day. Chimpanzees viewed the visual stimuli in two conditions, in which they heard either alarm calls or food grunts. Each condition comprised two trials presenting the snake and fruit videos on different sides (i.e., left/right). The order of conditions and the side on which the snake/fruit videos were presented were counterbalanced across chimpanzees. We repeated one trial for a female chimpanzee (Misaki) after the other trials were completed because she kept fixating on one location on the screen and appeared to be inattentive to the stimulus content.

Analyses

We used Tobii Studio v.3.4.8 to process the gaze data. The raw gaze recordings were filtered using a default Tobii Fixation Filter. We drew rectangular areas of interest (440 px × 440 px) for snake and fruit videos (Fig. 4.1c). Areas of interest were slightly larger than the video stimuli (400 px × 400 px) to accommodate minor recording errors.

For each trial, we calculated the proportion of time spent looking at snakes, namely snake gaze time divided by the total gaze time directed at either snake or fruit videos. A higher value indicates a longer gaze time to snake relative to fruit videos and a lower value indicates a longer gaze time to fruit relative to snake videos. A value of 0.5 indicates chance level (i.e., an equal gaze time to snake and fruit videos). Statistical analyses were performed using R v.4.0.0 (R Core Team 2020). We conducted a repeated-measures analysis of variance with condition (alarm call/food grunt) and location of the snake video (left/right) as independent variables, using the anovakun v.4.8.5 R function. For repeated measures, Cousineau–Morey–Baguley's difference-adjusted normalized confidence intervals (CIs; Baguley 2012) were calculated using anovakun v.4.8.5.

4.3.2 Results and Discussion

Chimpanzees viewed either snake or fruit videos for 10.4 ± 2.37 s (mean ± SD) in the alarm call condition and 11.3 ± 1.18 s in the food grunt condition. We found a significant main effect of condition, $F_{1,5} = 7.44$, $p = 0.041$, $\eta^2_G = 0.22$, with a higher proportion of snake gaze time during playback of alarm calls (mean = 0.44, 95% CI [0.37, 0.51]; two trials were averaged for each participant and the average and 95% CI of the six participants were calculated; Fig. 4.3) compared with playback of food grunts (mean = 0.28, 95% CI = [0.21, 0.36]). The other effects were not significant, video location: $F_{1, 5} = 0.31, p = 0.601, \eta^2_G = 0.01$; condition × video location: $F_{1, 5} = 2.52, p = 0.173, \eta^2_G = 0.07$.

This result suggests that chimpanzees looked at snake videos for longer when they heard alarm calls than when they heard food grunts, and/or that chimpanzees looked at fruit videos for longer when they heard food grunts than when they heard alarm calls. However, it remained unclear whether alarm calls caused gaze bias to snake videos or food grunts caused gaze bias to fruit videos, or both. To test this question, we conducted a follow-up experiment to examine the baseline gaze allocation to the fruit and snake videos when the chimpanzees were not hearing any auditory playbacks (in the online supplemental materials). This follow-up experiment suggested at least that the chimpanzees looked at the fruit videos for longer than the snake videos when there were no auditory playbacks. However, we could not strictly compare this result with that of experiment 1 because the order of conditions was not counterbalanced across participants. We addressed this issue in experiment 2.

4.4 Experiment 2

We tested another group of chimpanzees by counterbalancing the order of the three conditions (alarm call/food grunt/no-sound baseline). Note that we conducted several experiments with some procedural modifications before experiment 1, all of which produced null results (a brief overview of those experiments is provided in the online supplemental materials). We were concerned that those prior experiences might have somehow affected the results of experiment 1. Thus, we also aimed to ensure that different chimpanzees (with a less experience in those experiments) yielded the same results with identical procedures in experiment 2. Chimpanzees would be expected to look at the snake videos for longer when hearing alarm calls than when hearing no sound and to look at the fruit videos for longer when hearing food grunts than when hearing no sound.

4.4.1 Methods

Participants

Seven chimpanzees at the Primate Research Institute (PRI), Kyoto University, participated in experiment 2 (six females and one male, 19−43 years old, including estimated ages; Table 4.1). Chimpanzees lived in two adjacent groups with 11 individuals in total. Chimpanzees spent the daytime in outdoor enclosures (the largest one was open-air, approximately 700 m^2, accessible several days a week) with occasional access to indoor enclosures, in which they received meals and slept at night. These enclosures were equipped with complex structures such as climbing towers, platforms, hammocks, ropes, and/or small trees to allow chimpanzees to move and climb freely. Chimpanzees received meals comprising fresh vegetables, fruits, or nutritionally balanced biscuits three times a day. They had free access to water from the taps located in both outdoor and indoor enclosures. Chimpanzees were not deprived of food or water for the studies. The study depended on their voluntary participation. Animal husbandry and study protocols complied with the Guidelines for the Care and Use of Laboratory Primates by the Primate Research Institute, Kyoto University (2019-001).

Stimuli and Apparatus

Chimpanzees were invited into an indoor experimental booth (1.8 m × 2.15 m × 1.75 m) individually. Chimpanzees viewed stimuli through a transparent 2-cm-thick acrylic panel. We basically used the same stimuli and apparatuses as experiment 1. Nevertheless, sound volume may have been different because of differences in settings between the facilities such as different computers and experimental booths (the volume level was not recorded).

Procedures

Chimpanzees underwent one trial per day for a total of six trials (3 Auditory Stimuli × 2 Video Locations). Other procedures were the same as those of experiment 1. We repeated one trial for a female chimpanzee (Cleo) after other trials were completed because she left the eye tracker, presumably because she was afraid of the snake video. An experimenter error occurred in one trial with a male participant (Akira), and this trial was repeated on the same day (this might have happened with another participant [Pan] too).

4.4.2 Results and Discussion

Chimpanzees viewed either video type for 10.9 ± 0.92 s (mean ± SD) in the alarm call condition, 10.6 ± 1.56 s in the food grunt condition, and 10.8 ± 2.58 s in the no-sound condition. We found a significant main effect of condition, $F_{2,12} = 5.30, p = 0.023, \eta^2_G = 0.09$, whereas the other effects were not significant, video location: $F_{1,6} = 0.37, p = 0.563, \eta^2_G = 0.01$; Condition × Video Location: $F_{2,12} = 0.11, p = 0.896, \eta^2_G = 0.003$. The observed main effect of condition was explained by a higher proportion of snake gaze time when chimpanzees heard the alarm call (mean = 0.45; 95% CI [0.38, 0.53]) compared with when they heard no sound (mean = 0.32, 95% CI [0.27, 0.36]), $t_6 = 2.52, p = 0.045, d = 0.95$ (paired *t* test; Fig. 4.4), whereas there was no significant difference between when chimpanzees heard the food grunts (mean = 0.31, 95% CI [0.25, 0.36]) and when they heard no sound, $t_6 = -0.34, p = 0.747, d = -0.13$.

The results suggested that the playbacks of alarm calls caused a gaze bias to the snake videos, whereas playbacks of food grunts did not cause a gaze bias to the fruit videos. Thus, when chimpanzees heard the alarm calls, they may have spontaneously matched the alarm call with the image of snakes over the images of fruit. Alternatively, however, the gaze bias to snakes may have simply resulted from the negative affective state elicited by the calls independently of associations with snakes, for example via emotional contagion (Briefer 2018; de Waal 2008; Hatfield et al. 1992). The matched negative affective states may have biased the chimpanzees' attention to potential threats via affect-driven attention biases (reviewed in Crump et al. 2018). We addressed this issue in experiment 3.

4.5 Experiment 3

We examined whether presenting screams, an irrelevant call with negative valence, biased chimpanzees' gaze in the same way as an alarm call. Specifically, we tested the same two groups of chimpanzees using the same visual stimuli as experiments 1 and 2 coupled with either screams or no sound. Chimpanzees would not be expected to look at snake videos for longer when hearing screams than when hearing no sound.

4.5.1 Methods

Participants

The same 13 chimpanzees included in experiments 1 and 2 participated in experiment 3 (Table 4.1).

Stimuli and apparatus

Auditory stimuli were screams (although a finer categorization [as did in Goodall 1986, Chapter 6] was not made). We recorded screams (0.8 s and 0.9 s were clipped from video recordings and are shown as spectrograms in the right column of Fig. 4.1b) from two of the female chimpanzee participants at KS during possible agonistic interactions in an outdoor enclosure (in one case, the interaction was not clearly observed). The 0.8-s and 0.9-s calls were played repeatedly 10 times. The calls were distributed over the entire duration of the 12-s video at arbitrary intervals (Video S4.3 in the online supplemental materials). The same composition was used across trials. We presented the same visual stimuli as experiments 1 and 2 (although the renditions might have differed slightly from those experiments, and between facilities, due to possible differences in setting and version of Xmedia Recode software) coupled with screams or no sound (although the exact volume level of playback was not recorded at the time, it was estimated to be approximately 70 dBA by post hoc measurement at KS; not recorded at PRI). As with experiments 1 and 2, each auditory condition comprised of two trials with different location of videos (left/right), and thus experiment 3 comprised of four trials in total.

Procedures

We used the same procedures as those in experiments 1 and 2. Two trials for a female chimpanzee (Misaki) were repeated after all other trials were completed because she kept fixating on one location on the screen and appeared to be inattentive to the stimulus content. One trial for a male chimpanzee (Zamba) was also repeated because he substantially changed his posture and viewing angle during the trial.

4.5.2 Results and Discussion

Chimpanzees viewed either video type for 10.8 ± 1.63 s (mean ± SD) in the scream condition and 10.7 ± 1.33 s in the no-sound condition. We found no significant effects, condition: $F_{1, 12} = 0.37$, $p = 0.555$, $\eta^2_G = 0.007$; video location: $F_{1, 12} = 0.39$, $p = 0.544$, $\eta^2_G = 0.007$; Condition × Video Location: $F_{1, 12} = 0.06$, $p = 0.813$, $\eta^2_G = 0.0009$ (scream: mean = 0.33, 95% CI [0.28, 0.39]; no sound: mean = 0.30, 95% CI [0.25, 0.36]; Fig. 4.5). Note that including group (i.e., KS/PRI) as a between-participants variable did not change the pattern of results.

These findings suggest that chimpanzees did not look at the images of snakes just because the alarm call evoked negative affective states and thereby biased chimpanzees' attention to threatening stimuli via affect-driven attention biases. Nonetheless, we could not completely rule out the possibility that prior experiences with similar stimuli may have affected the results. That is, chimpanzees had experienced other conditions (experiments 1 for KS chimpanzees; experiment 2 for PRI chimpanzees) before the scream condition (experiment 3), and those prior experiences with stimuli might have affected the results of experiment 3, including the absence of the gaze bias to snake stimuli. However, this possibility seems unlikely to explain all of the results, considering that the playback of alarm calls elicited a gaze bias in KS chimpanzees (experiment 1), who had experienced similar experiments prior to experiment 1 (in the online supplemental materials), as well as in PRI chimpanzees (experiment 2), who had only a few such experiences, which suggests that prior experiences had only a minimal impact.

4.6 General Discussion

Chimpanzees looked at videos of snakes for longer when they heard alarm calls compared with when they heard food grunts (experiment 1) or no sound (experiment 2). These results indicated that when chimpanzees heard alarm calls, they spontaneously matched the call with images of a potential threat over nonthreat-related images. Experiment 3 showed that playback of screams did not bias chimpanzees' gaze toward the snake video. Therefore, chimpanzees may not have looked at the images of snakes just because the alarm call evoked negative affective states, independently of associations with snakes (e.g., emotional contagion: Briefer 2018), which biased chimpanzees' attention to threatening stimuli via affect-driven attention biases. These results are in line with the interpretation given by Shibasaki et al. (2014) that audience animals recollected a representation of a snake through an association.

However, several limitations may have affected our results and interpretations. First, chimpanzees did not look at the fruit videos for longer when they heard food grunts compared with baseline (experiment 2). This finding may have occurred simply because chimpanzees were already biased to look at the fruit videos, as observed in the no-sound baseline condition in experiment 2 (and in a follow-up study of the findings of experiment 1, as summarized in the online supplemental materials). An overall bias toward the fruit videos may have made it difficult to detect further gaze bias in the same direction. Second, it remains unclear whether chimpanzees could recall the image of a potential threat without any presented images. We presented participants with two images side-by-side while playing back calls simultaneously. Thus, it is possible that chimpanzees may have compared the presented options and inferred only the most relevant item among them (Adachi et al. 2007; for a relevant finding with human infants, see Wu et al. 2017). Future experiments in which an auditory stimulus is played before showing the visual stimulus may be useful to more strictly examine whether calls evoke a representation of a specific object (Suzuki 2016, 2018).

Third, although the results revealed that chimpanzees did not match the alarm call with the image of snakes simply based on the negative valence of the stimuli, it remains possible that more subtle differences in affect biased their attention via emotional contagion. That is, alarm calls and screams might have evoked subtly different affective states, if any, which may be closely matched with those of the respective callers, thereby biasing chimpanzees' attention toward the items that elicited the calls. Currently, however, there seems to be little evidence suggesting such fine-tuned state-matching via vocal contagion in nonhuman animals (previous findings are reviewed in Briefer 2018). Nonetheless, further studies are necessary to test this possibility.

Fourth, it should be noted that we used a single composition for each class of auditory and visual stimuli, although each consisted of several exemplars (i.e., four for snake and fruit videos; two for alarm call, food call, and scream). Thus, this experimental design, with the corresponding statistical analyses, suffered from the issue of pseudoreplication (Hurlbert 1984; Kroodsma et al. 2001; McGregor 2000). Thus, we could not infer with confidence to what extent our results are generalizable to the objects, or the call types in general (e.g., Kroodsma 1989a, 1989b; McGregor 2000; Wiley 2003). Ideally, future studies should use a sufficiently large number of exemplars for each class of stimuli. In an additional analysis presented in the online supplemental materials, we examined the effects of different visual exemplars on the observed gaze responses to snakes. Specifically, we divided each trial into four time bins, each approximately corresponding to each 3-s video pair (stills are shown in Fi. 4.1a), and compared the proportion of time spent looking at snake videos across conditions and time bins via repeated-measures analysis of variance. These analyses indicated that the proportion of snake gaze time differed across time bins. However, the differential gaze responses evoked by alarm calls were not due to a particular time bin (and possibly video pair). We did not carry out a similar analysis for the auditory stimuli because the two calls were played with short intervals between each other and sometimes even overlapped, which hindered segmentation of each trial into bins per call sequence (Videos S4.1−4.3 in the online supplemental materials).

In conclusion, our results revealed that chimpanzees spontaneously matched an alarm call with threat-related over nonthreat-related stimuli in a cross-modal preferential-looking experiment. It is possible that such audiovisual cross-modal associations indicate the existence of mental representation of the referent that guides flexible adjustments of behavior in both callers and audiences. Further studies will be necessary to test this possibility in depth.

4.7 Acknowledgments

The authors thank the staff at Kumamoto Sanctuary and members of the Language and Intelligence group at the Primate Research Institute for their cooperation in data collection, Mr. Masaya Fukuda for his cooperation in material preparation, Tetsuro Matsuzawa and the Great Ape Information Network for financial support, and Benjamin Knight from Edanz Group (https://jp.edanz.com/ac) for language editing of a draft. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology through a Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for JSPS Fellows to Yutaro Sato (Grant 19J22889); a Grant-in-Aid for Scientific Research (B) and a Grant-in-Aid for Scientific Research on Innovative Areas to Fumihiro Kano (Grants 19H01772, 20H05000); a Grant-in-Aid for Scientific Research (S) and a Grant-in-Aid for Scientific Research on Innovative Areas to Masaki Tomonaga (Grants 15H05709, 18H04194); a Grant-in-Aid for Scientific Research (A) and a Grant-in-Aid for Scientific Research on Innovative Areas to Satoshi Hirata (Grants 26245069, 18H05524); a Grant-in-Aid for Specially Promoted Research to Tetsuro Matsuzawa (Grant 16H06283); a Program for Leading Graduate Schools to Tetsuro Matsuzawa (LDG-U04); the Great Ape Information Network; and the Cooperative Research Program of Primate Research Institute, Kyoto University (2019-A-1).

4.8 References

Adachi I, Kuwahata H, Fujita K (2007) Dogs recall their owner's face upon hearing the owner's voice. Anim Cogn 10(1):17–21. https://doi.org/10.1007/s10071-006-0025-8

Baguley T (2012) Calculating and graphing within-subject confidence intervals for ANOVA. Behav Res Methods 44(1):158–175. https://doi.org/10.3758/s13428-011-0123-7

Briefer EF (2018) Vocal contagion of emotions in non-human animals. Proc Royal Soc B Biol Sci 285(1873):20172783. https://doi.org/10.1098/rspb.2017.2783

Brosnan SF, de Waal FBM (2000) Regulation of vocal output by chimpanzees finding food in the presence or absence of an audience. Evol Commun 4(2):211–224. https://doi.org/10.1075/eoc.4.2.05bro

Clay Z, Zuberbühler K (2011) Bonobos extract meaning from call sequences. PLoS ONE 6(4):e18786. https://doi.org/10.1371/journal.pone.0018786

Crockford C, Boesch C (2003) Context-specific calls in wild chimpanzees, *Pan troglodytes verus*: analysis of barks. Anim Behav 66(1):115–125. https://doi.org/10.1006/anbe.2003.2166

Crockford C, Wittig RM, Mundry R, Zuberbühler K (2012) Wild chimpanzees inform ignorant group members of danger. Curr Biol 22(2):142–146. https://doi.org/10.1016/j.cub.2011.11.053

Crockford C, Wittig RM, Zuberbühler K (2015) An intentional vocalization draws others' attention: a playback experiment with wild chimpanzees. Anim Cogn 18(3):581–591. https://doi.org/10.1007/s10071-014-0827-z

Crockford C, Wittig RM, Zuberbühler K (2017) Vocalizing in chimpanzees is influenced by social-cognitive processes. Sci Adv 3(11):e1701742. https://doi.org/10.1126/sciadv.1701742

Crump A, Arnott G, Bethell EJ (2018) Affect-driven attention biases as animal welfare indicators: review and methods. Animals 8(8):136. https://doi.org/10.3390/ani8080136

DeLoache JS, LoBue V (2009) The narrow fellow in the grass: human infants associate snakes and fear. Dev Sci 12(1):201–207. https://doi.org/10.1111/j.1467-7687.2008.00753.x

de Waal FBM (2008) Putting the altruism back into altruism: the evolution of empathy. Annu Rev Psychol 59:279–300. https://doi.org/10.1146/annurev.psych.59.103006.093625

Dezecache G, Berthet M (2018) Working hypotheses on the meaning of general alarm calls. Anim Behav 142:113–118. https://doi.org/10.1016/j.anbehav.2018.06.008

Dezecache G, Crockford C, Zuberbühler K (2019) The development of communication in alarm contexts in wild chimpanzees. Behav Ecol Sociobiol 73(8):1–9. https://doi.org/10.1007/s00265-019-2716-6

Evans CS, Evans L (2007) Representational signalling in birds. Biol Lett 3(1):8–11. https://doi.org/10.1098/rsbl.2006.0561

Fedurek P, Slocombe KE (2013) The social function of food-associated calls in male chimpanzees. Am J Primatol 75(7):726–739. https://doi.org/10.1002/ajp.22122

Gibbons C (2007) The referentiality of chimpanzee vocal signaling: behavioral and acoustic analysis of food barks. Electronic Thesis or Dissertation, Ohio State University.

Girard-Buttoz C, Surbeck M, Samuni L et al (2020) Information transfer efficiency differs in wild chimpanzees and bonobos, but not social cognition. Proc Royal Soc B Biol Sci 287(1929):20200523. https://doi.org/10.1098/rspb.2020.0523

Goodall J (1986) The chimpanzees of Gombe: patterns of behavior. Harvard University Press, Cambridge, pp 117–145

Hallberg KI, Nelson DA, Boysen ST (2003) Representational vocal signaling in the chimpanzee. In: de Waal FBM, Tyack PL (eds) Animal social complexity. Intelligence, culture, and individualized societies. Harvard University Press, Cambridge, pp 317–321

Hatfield E, Cacioppo JT, Rapson RL (1992) Primitive emotional contagion. In: Clark MS (ed) Review of personality and social psychology, vol 14. Emotion and social behavior. Sage, Thousand Oaks, pp 151–177

Hauser MD, Teixidor P, Field L, Flaherty R (1993) Food-elicited cells in chimpanzees: effects of food quantity and divisibility. Anim Behav 45(4):817−819. https://doi.org/10.1006/anbe.1993.1096

Hauser MD, Wrangham RW (1987) Manipulation of food calls in captive chimpanzees: a preliminary report. Folia Primatol 48(3–4):207–210. https://doi.org/10.1159/000156298

Hopper LM, Gulli RA, Howard LH, Kano F, Krupenye C, Ryan AM, Paukner A (2020) The application of noninvasive, restraint-free eye-tracking methods for use with nonhuman primates. Behav Res Methods 53(3):1003−1030. https://doi.org/10.3758/s13428-020-01465-6

Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecol Monogr 54(2):187−211. https://doi.org/10.2307/1942661

Idani G, Hirata S (2006) Studies at the Great Ape Research Institute. In: Washburn DA (ed) Primate perspectives on behavior and cognition. American Psychological Association, Washington, pp 29–36

Kalan AK, Boesch C (2015) Audience effects in chimpanzee food calls and their potential for recruiting others. Behav Ecol Sociobiol 69(10):1701–1712. https://doi.org/10.1007/s00265-015-1982-1

Kalan AK, Mundry R, Boesch C (2015) Wild chimpanzees modify food call structure with respect to tree size for a particular fruit species. Anim Behav 101:1–9. https://doi.org/10.1016/j.anbehav.2014.12.011

Kroodsma DE (1989a) Inappropriate experimental designs impede progress in bioacoustic research: a reply. Anim Behav 38(4):717–719. https://doi.org/10.1016/S0003-3472(89)80020-X

Kroodsma DE (1989b). Suggested experimental designs for song playbacks. Anim Behav 37(4):600–609. https://doi.org/10.1016/0003-3472(89)90039-0

Kroodsma DE, Byers BE, Goodale E, Johnson S, Liu, WC (2001) Pseudoreplication in playback experiments, revisited a decade later. Anim Behav 61(5):1029–1033. https://doi.org/10.1006/anbe.2000.1676

Leroux M, Bosshard AB, Chandia B, Manser A, Zuberbühler K, Townsend SW (2021) Chimpanzees combine pant hoots with food calls into larger structures. Anim Behav 179:41–50. https://doi.org/10.1016/j.anbehav.2021.06.026

Macedonia JM, Evans CS (1993) Essay on contemporary issues in ethology: variation among mammalian alarm call systems and the problem of meaning in animal signals. Ethology 93(3):177–197. https://doi.org/10.1111/j.1439-0310.1993.tb00988.x

Marler P, Tenaza R (1977) Signaling behavior of apes with special reference to vocalization. In: Sebeok TA (ed) How animals communicate. Indiana University Press, Bloomington, pp 965–1033

Matsuzawa T (2006) Sociocognitive development in chimpanzees: a synthesis of laboratory work and fieldwork. In: Matsuzawa T, Tomonaga M, Tanaka M (eds) Cognitive development in chimpanzees. Springer, Tokyo, pp 3–33. https://doi.org/10.1007/4-431-30248-4_1

McGregor PK (2000) Playback experiments: design and analysis. Acta Ethol 3(1):3–8. https://doi.org/10.1007/s102110000023

O’Bryan LR (2015) The socioecology of chimpanzee foraging and food-associated calling behavior. Doctoral dissertation, University of Minnesota.

O’Bryan LR, Lambeth SP, Schapiro SJ, Wilson ML (2021) Playbacks of food-associated calls attract chimpanzees towards known food patches in a captive setting. Primates 62(6):905–918. https://doi.org/10.1007/s10329-021-00936-4

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.r-project.org/

Schel AM, Townsend SW, Machanda Z, Zuberbühler K, Slocombe KE (2013) Chimpanzee alarm call production meets key criteria for intentionality. PLoS ONE 8(10):e76674. https://doi.org/10.1371/journal.pone.0076674

Seyfarth RM, Cheney DL (1990) The assessment by vervet monkeys of their own and another species’ alarm calls. Anim Behav 40(4):754–764. https://doi.org/10.1016/S0003-3472(05)80704-3

Seyfarth RM, Cheney DL (2003) Meaning and emotion in animal vocalizations. Ann NY Acad Sci 1000(1):32–55. https://doi.org/10.1196/annals.1280.004

Shibasaki M, Nagumo S, Koda H (2014) Japanese monkeys (*Macaca fuscata*) spontaneously associate alarm calls with snakes appearing in the left visual field. J Comp Psychol 128(3):332–335. https://doi.org/10.1037/a0036049

Slocombe KE, Kaller T, Turman L, Townsend SW, Papworth S, Squibbs P, Zuberbühler K (2010) Production of food-associated calls in wild male chimpanzees is dependent on the composition of the audience. Behav Ecol Sociobiol 64(12):1959–1966. https://doi.org/10.1007/s00265-010-1006-0

Slocombe KE, Zuberbühler K (2005) Functionally referential communication in a chimpanzee. Curr Biol 15(19):1779–1784. https://doi.org/10.1016/j.cub.2005.08.068

Slocombe KE, Zuberbühler K (2006) Food-associated calls in chimpanzees: responses to food types or food preferences? Anim Behav 72(5):989–999. https://doi.org/10.1016/j.anbehav.2006.01.030

Suzuki TN (2016) Semantic communication in birds: evidence from field research over the past two decades. Ecol Res 31(3):307–319. https://doi.org/10.1007/s11284-016-1339-x

Suzuki TN (2018) Alarm calls evoke a visual search image of a predator in birds. Proc Natl Acad Sci USA 115(7):1541–1545. https://doi.org/10.1073/pnas.1718884115

Suzuki TN (2020) Other species' alarm calls evoke a predator-specific search image in birds. Curr Biol 30(13):2616–2620.e2. https://doi.org/10.1016/j.cub.2020.04.062

Townsend SW, Manser MB (2013) Functionally referential communication in mammals: the past, present and the future. Ethology 119(1):1–11. https://doi.org/10.1111/eth.12015

Wiley RH (2003) Is there an ideal behavioural experiment? Anim Behav 66(3):585–588. https://doi.org/10.1006/anbe.2003.2231

Wheeler BC, Fischer J (2012) Functionally referential signals: a promising paradigm whose time has passed. Evol Anthropol 21(5):195–205. https://doi.org/10.1002/evan.21319

Wu Y, Muentener P, Schulz LE (2017) One- to four-year-olds connect diverse positive emotional vocalizations to their probable causes. Proc Natl Acad Sci USA 114(45): 11896–11901. https://doi.org/10.1073/pnas.1707715114

Zuberbühler K (2000) Causal cognition in a non-human primate: field playback experiments with Diana monkeys. Cognition 76(3):195–207. https://doi.org/10.1016/S0010-0277(00)00079-2

Zuberbühler K, Cheney DL, Seyfarth RM (1999) Conceptual semantics in a nonhuman primate. J Comp Psychol 113(1):33–42. https://doi.org/10.1037/0735-7036.113.1.33

4.9 Tables and Figures

Table 4.1 Chimpanzee participant information.

Site	Name	Sex	Age	GAIN ID[a]
Kumamoto Sanctuary (Experiments 1 and 3)	Hatsuka	F	11	0704
	Iroha	F	10–11	0708
	Misaki	F	20	0593
	Mizuki	F	22	0559
	Natsuki	F	14	0677
	Zamba	M	24	0543
Primate Research Institute (Experiments 2 and 3)	Ai	F	43[b]	0434
	Akira	M	43[b]	0435
	Chloe	F	38	0441
	Cleo	F	19	0609
	Pal	F	19	0611
	Pan	F	35	0440
	Pendesa	F	42	0095

Note. In the column Sex, “F” and “M” denote “Female” and “Male,” respectively.

[a] GAIN ID is the identification from the Great Ape Information Network (https://shigen.nig.ac.jp/gain/index.jsp). [b] Estimated ages.

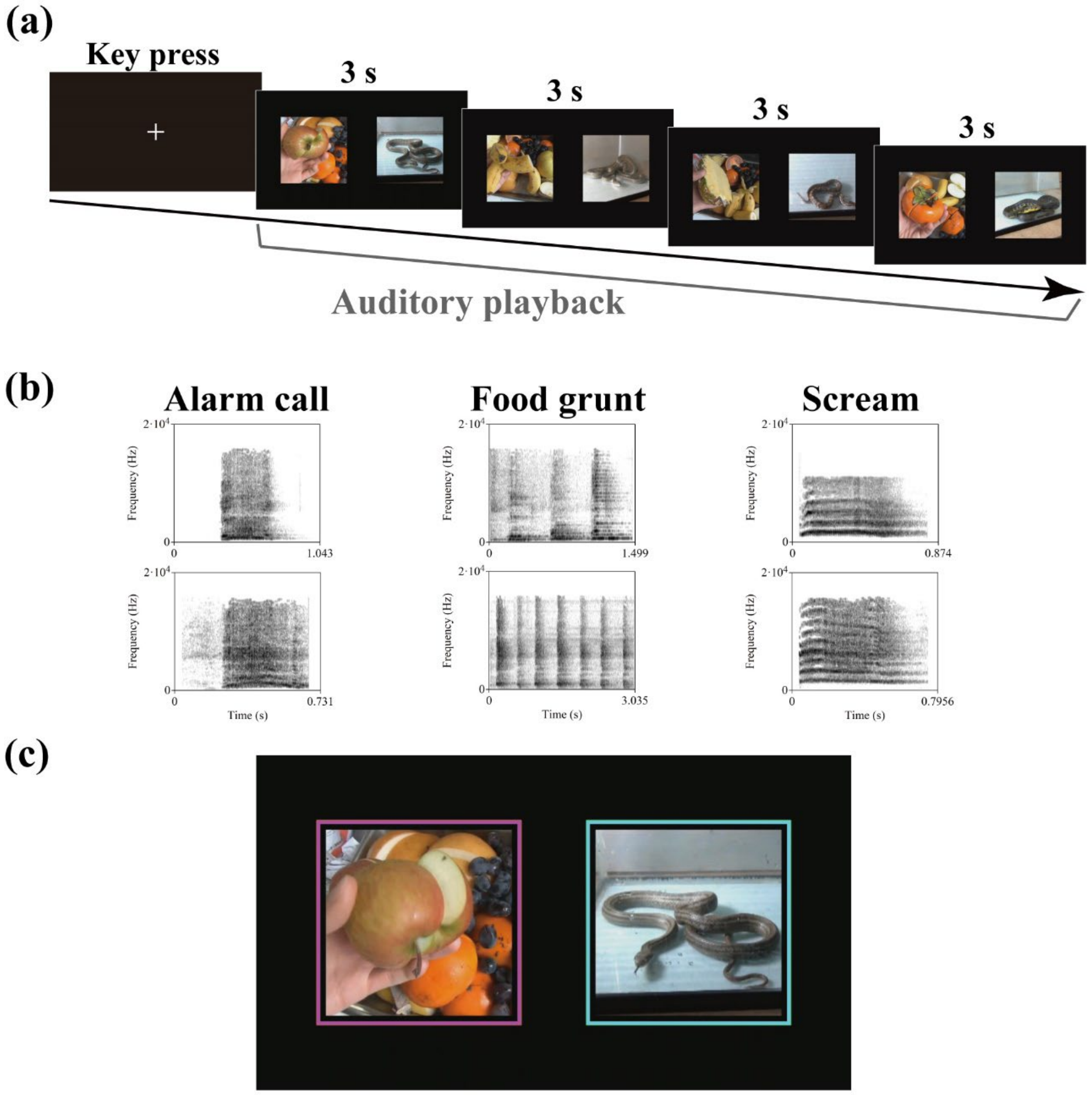

Fig. 4.1 Illustrations for materials and method.

Note. **a** Trial flow. **b** Spectrograms of auditory stimuli. We used PRAAT 6.1.04 to construct spectrograms with the following parameters (window length = 0.005 s, maximum frequency = 20,000 Hz, time step = 0.002 s, frequency step = 20 Hz, window shape = Gaussian, dynamic range = 50 dB). **c** The magenta (dark gray) and cyan (light gray) squares show the areas of interest in analysis and are included in the figure for illustrative purposes only.

Fig. 4.2 Experimental setting at Kumamoto Sanctuary.

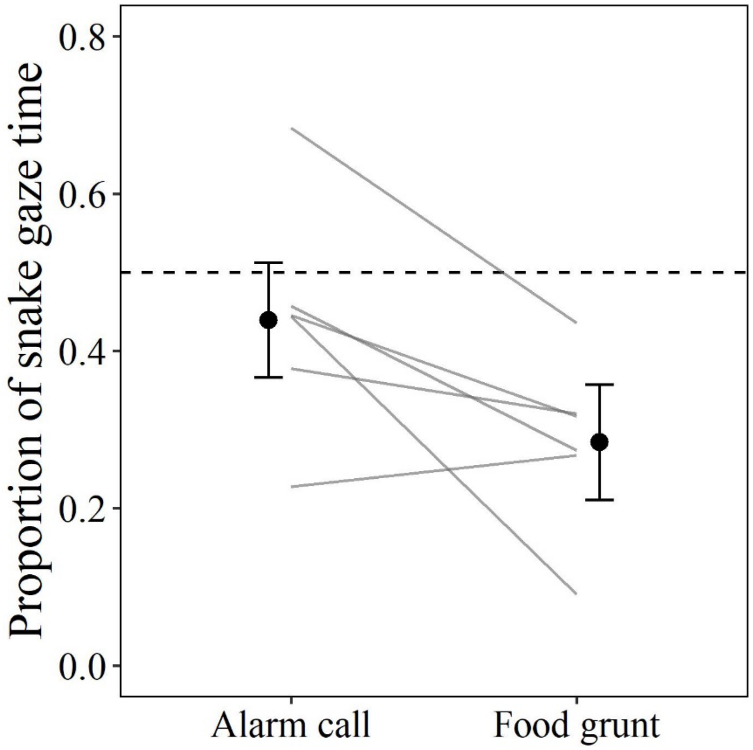

Fig. 4.3 Proportion of time spent looking toward the snake video stimulus in experiment 1 (*n* = 6).

Note. The gray lines represent the individual data (average of two trials). The black dots represent the mean, and error bars represent the 95% CI.

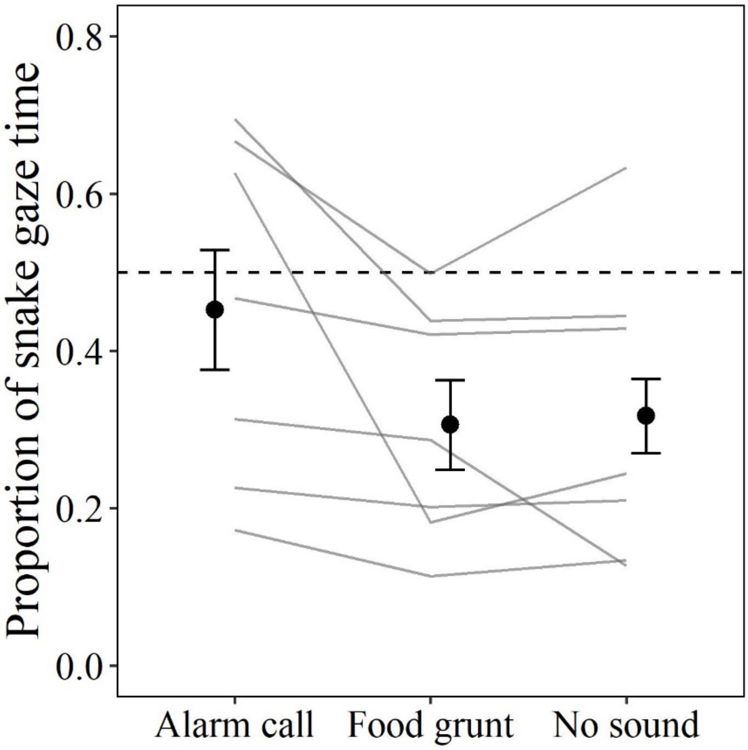

Fig. 4.4 Proportion of time spent looking toward the snake video stimulus in experiment 1 (*n* = 7).

Note. The gray lines represent the individual data (average of two trials). The black dots represent the mean, and error bars represent the 95% CI.

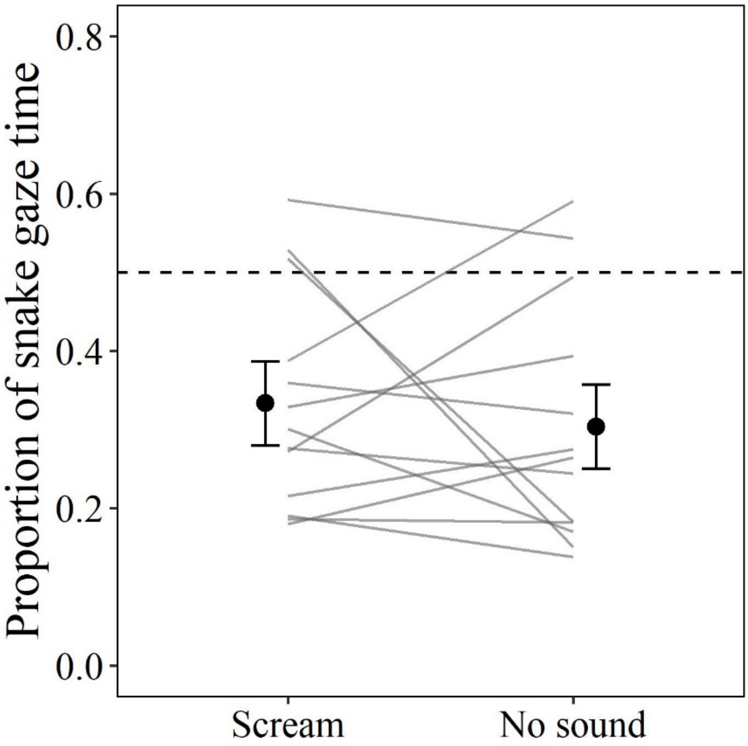

Fig. 4.5 Proportion of time spent looking toward snake video in experiment 3 (*n* = 13, data from two groups were pooled [Kumamoto Sanctuary and Primate Research Institute]).

Note. The gray lines represent the individual data (average of two trials). The black dots represent the mean, and the error bars represent the 95% CI.

4.10 Supplementary Materials

Supplementary Videos

Video S4.1: Video stimulus in the alarm call condition.

Video S4.2: Video stimulus in the food grunt condition.

Video S4.3: Video stimulus in the scream condition.

Time Bin Analysis

The visual stimuli were comprised of four 3-s video pairs (i.e., fruit vs. snake) presented successively in a trial, in the same order across trials. To examine the effects of particular video pairs, we conducted additional analyses, in which the whole stimulus duration (i.e., 12 s) was divided into four time bins, which roughly corresponded to the presentation of each video pair. Specifically, using Tobii Studio software, we specified the following time windows: 0–3 s, 3–6 s, 6–9 s, and 9 s–stimulus end (the last bin was slightly longer than 3 s because of errors). We then calculated the proportion of time spent looking at the snake stimulus in each time bin. Using the anovakun v.4.8.5 R function, we ran repeated-measures analyses of variance with condition (i.e., auditory playback), location of the snake video (left/right), and time bin (first/second/third/fourth) as independent variables. The sample sizes were not sufficiently large for sphericity tests to be valid. As a compromise, we adjusted the degrees of freedom irrespective of sphericity tests (as recommended by Howell, 2010, Chapter 14). To achieve this, we used Huynh–Feldt–Lecoutre's epsilon (note that omitting the adjustment did not alter the overall conclusion). Multiple comparisons were performed using Shaffer's modified sequentially rejective Bonferroni procedure.

Regarding experiment 1, we found significant main effects of condition ($F_{1, 5}$ = 18.32, $p = 0.008$, $\eta^2_G = 0.13$) and time bin ($F_{2.98, 14.9} = 12.39$, $p < 0.001$, $\eta^2_G = 0.21$). Other effects were not significant (ps > 0.05) (Table S4.1). Multiple comparisons indicated that the proportion of snake gaze time was significantly smaller in the first vs. third ($t_5 = 5.97$, p_{adj} [adjusted p-value] = 0.011) and second vs. third bins ($t_5 = 5.44$, $p_{\text{adj}} = 0.011$), while differences between other bins were not significant (p_{adj}s > 0.05) (Fig. S4.1). These results suggest that chimpanzees allocated their gaze to snake and fruit videos differently depending on time bins or video pairs and that they looked at snake videos for longer overall when hearing alarm calls vs. food grunts.

Regarding experiment 2, the data of two participants were omitted from the analysis because of missing values. We found a significant main effect of time bin ($F_{1.36, 5.45} = 10.26$, $p = 0.018$, $\eta^2_G = 0.11$). Other effects were not significant (ps > 0.05) (Table S4.2). Multiple comparisons indicated that the proportion of snake gaze time was significantly larger in the first vs. second bins ($t_4 = 6.90$, $p_{\text{adj}} = 0.007$) and smaller in the second vs. third bins ($t_4 = 13.19$, $p_{\text{adj}} = 0.001$). Other effects were not significant (p_{adj}s > 0.05) (Fig. S4.2). In line with the results of experiment 1, these results suggest that chimpanzees allocate their gaze to snake and fruit videos differently depending on time bins or video pairs. Although the main effect of condition was not significant, it seems unlikely that the effect of alarm calls on the bias toward snake videos reported in the main text was caused by a particular bin or video pair.

Regarding experiment 3, the data of one participant were omitted from the analysis because of a missing value. We found a significant main effect of time bin ($F_{1.86, 20.46} = 13.95$, $p < 0.001$, $\eta^2_G = 0.14$) and a significant interaction of condition by time bin ($F_{2.99, 32.89} = 4.20$, $p = 0.013$, $\eta^2_G = 0.04$). Other effects were not significant (ps > 0.05) (Table S4.3). Simple effects tests of the “condition × time bin” interaction revealed that, in the second bin, the proportion of snake gaze time was larger when hearing screams compared with hearing no sound ($F_{1, 11} = 4.89$, $p = 0.049$, $\eta^2_G = 0.07$). In the third bin, in contrast, the proportion of snake gaze time was smaller when hearing screams compared with hearing no sound ($F_{1, 11} = 6.61$, $p = 0.026$, $\eta^2_G = 0.07$) (Fig. S4.3). No significant effects were found for the first and fourth bins (*p*s > 0.05). It remains unclear why the scream playback caused gaze bias in the opposite direction between the second and third bins. Nevertheless, the results suggest that the effect of screams on gaze bias, if any, was smaller than the effect of alarm calls on gaze bias.

Table S4.1. Repeated-measures analysis of variance for experiment 1

Source	*SS*	*df*	*MS*	*F*-ratio	*p*-value	η^2_G
Condition	0.71	1, 5	0.71	18.32	0.008	0.13
Location	0.09	1, 5	0.09	0.57	0.484	0.02
Time bin	1.26	2.98, 14.9	0.42	12.39	< 0.001	0.21
Condition × Location	0.21	1, 5	0.21	6.53	0.051	0.04
Location × Time bin	0.16	2.93, 14.64	0.05	0.59	0.625	0.03
Condition × Time bin	0.09	2.69, 13.47	0.03	0.90	0.457	0.02
Condition × Location × Time bin	0.16	3, 5	0.05	1.87	0.178	0.03

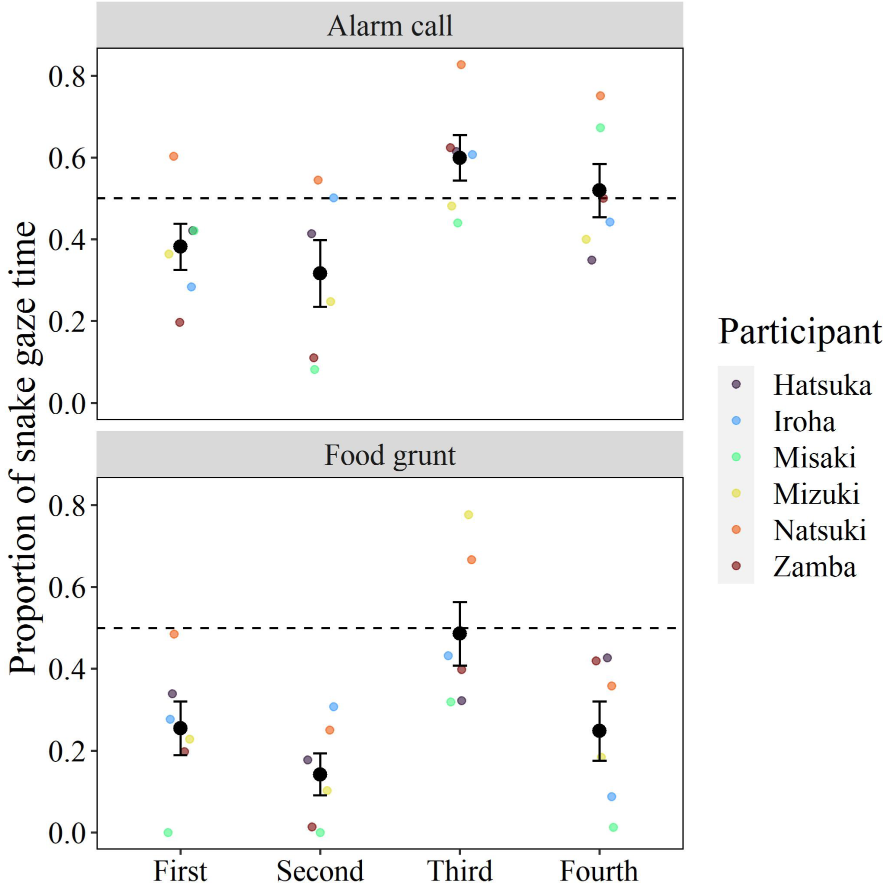

Fig. S4.1 Proportion of time spent looking toward snake videos in each time bin in experiment 1 ($n = 6$). The colored dots represent the individual data (average of two trials), jittered horizontally for illustrative purposes. The black dots represent the mean, and error bars represent the standard error of the mean. The dashed horizontal line indicates a value of 0.5.

Table S4.2 Repeated-measures analysis of variance for experiment 2.

Source	*SS*	*df*	*MS*	*F*-ratio	*p*-value	η^2_G
Condition	0.73	1.72, 6.87	0.42	4.04	0.073	0.09
Location	0.24	1, 4	0.24	1.16	0.341	0.03
Time bin	0.86	1.36, 5.45	0.63	10.26	0.018	0.11
Condition × Location	0.04	2, 8	0.02	0.11	0.895	0.005
Location × Time bin	0.30	2.67, 10.68	0.11	2.30	0.140	0.04
Condition × Time bin	0.11	3.42, 13.67	0.03	0.40	0.777	0.01
Condition × Location × Time bin	0.42	6, 24	0.07	2.08	0.094	0.06

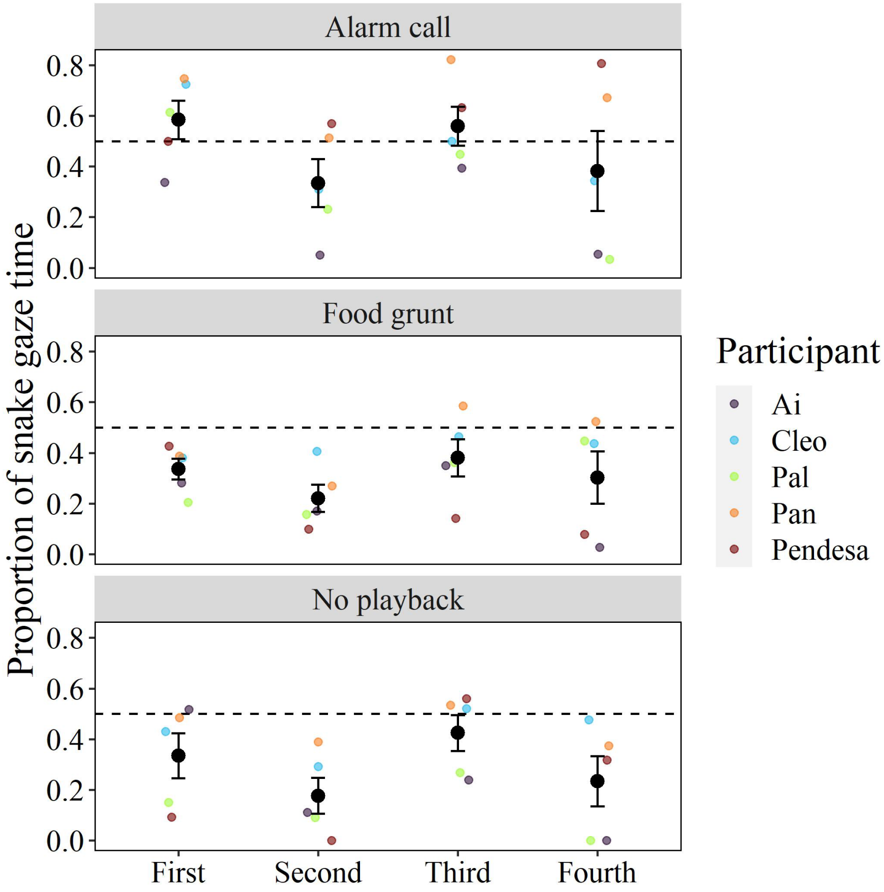

Fig. S4.2 Proportion of time spent looking toward snake videos in each time bin in Experiment 2 ($n = 5$). The colored dots represent the individual data (average of two trials), jittered horizontally for illustrative purposes. The black dots represent the mean, and error bars represent the standard error of the mean. The dashed horizontal line indicates a value of 0.5.

Table S4.3. Repeated-measures analysis of variance for experiment 3.

Source	*SS*	*df*	*MS*	*F*-ratio	*p*-value	η^2_G
Condition	0.01	1, 11	0.01	0.10	0.754	0.001
Location	0.14	1, 11	0.14	1.22	0.294	0.01
Time bin	1.93	1.86, 20.46	1.04	13.95	< 0.001	0.14
Condition × Location	0.0006	1, 11	0.0006	0.005	0.943	0.0001
Location × Time bin	0.04	2.37, 26.03	0.02	0.24	0.820	0.003
Condition × Time bin	0.48	2.99, 32.89	0.16	4.20	0.013	0.04
Condition × Location × Time bin	0.08	2.36, 26.01	0.04	0.81	0.472	0.007

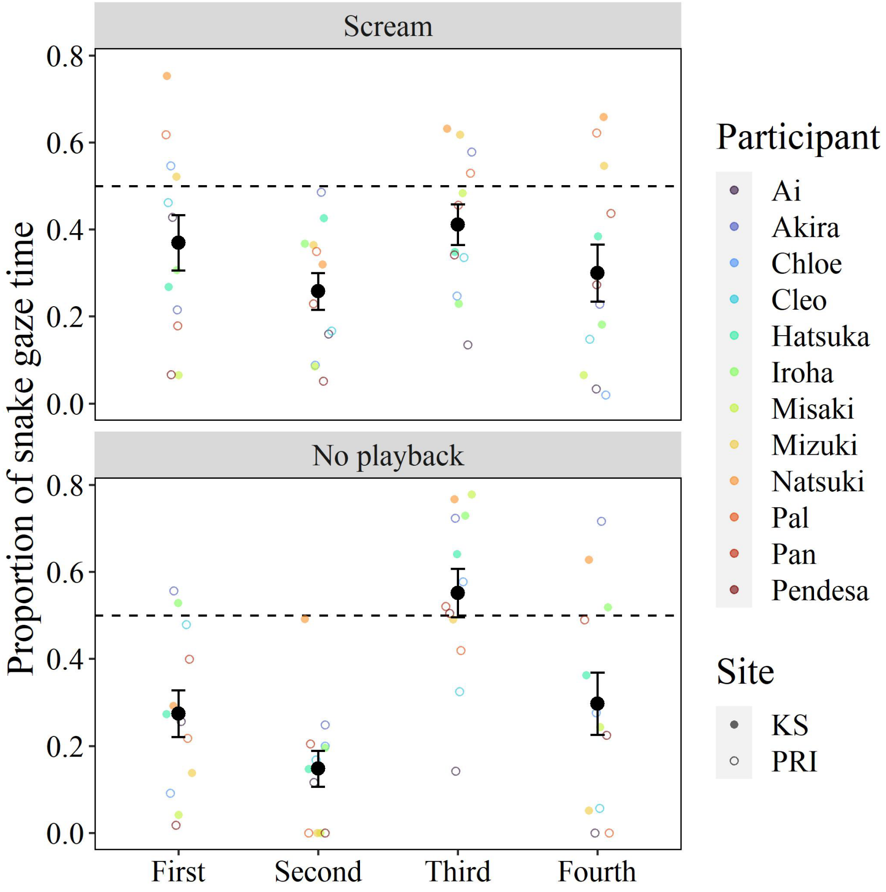

Fig. S4.3 Proportion of time spent looking toward snake videos in each time bin in experiment 1 ($n = 12$, data from two groups was pooled [Kumamoto Sanctuary and Primate Research Institute]). The colored dots represent the individual data (average of two trials), jittered horizontally for illustrative purposes. The black dots represent the mean, and error bars represent the standard error of the mean. The dashed horizontal line indicates a value of 0.5.

A Follow-Up Experiment for Experiment 1

We sought to follow up experiment 1 by examining chimpanzees' gaze to the videos of snakes and fruit without auditory playbacks, to provide a measure of baseline gaze allocation to the visual stimuli. Specifically, we presented the same chimpanzees with the same visual stimuli as experiment 1 but with no sound, which served as a baseline condition.

Methods

The same six chimpanzees in experiment 1 participated in this experiment (Table 4.1). We presented the same visual stimuli with no sound. Chimpanzees underwent one trial per day for a total of two trials (two patterns of video locations). Other procedures were the same as those in experiment 1.

Results and Discussion

Chimpanzees viewed either video type for 10.5 ± 1.09 s (mean ± SD). The proportion of snake gaze time was significantly smaller than chance level (vs. 0.5; mean = 0.37, 95% CI = [0.33, 0.41]; one-sample t test: $t_5 = -8.65$, $p < 0.001$, $d = -3.53$; Fig. S4.4). This result indicates that chimpanzees were already biased to look at the video of fruit.

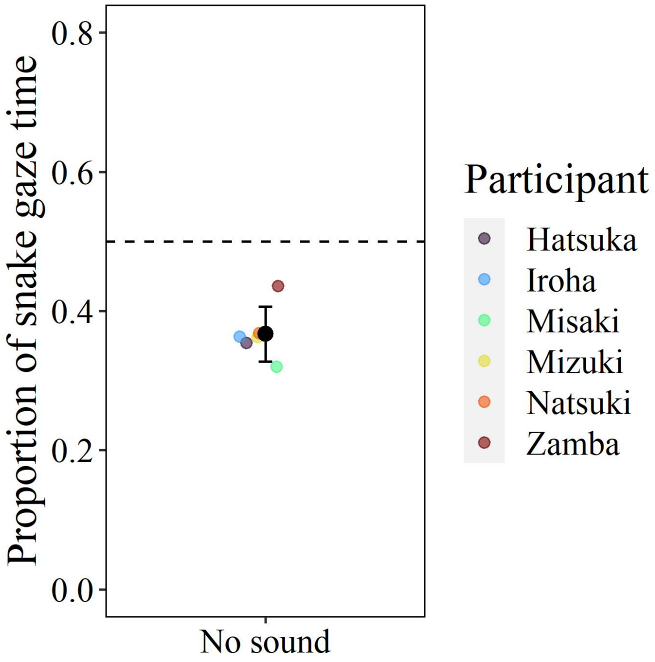

Fig. S4.4 Proportion of time spent looking toward snake videos (*n* = 6). The colored dots represent the individual data (average of two trials). The black dots represent the mean, and error bars represent 95% CI. The dashed line indicates a value of 0.5. The colored dots are jittered horizontally for illustrative purposes.

Preceding Experiments

We conducted several experiments before conducting the current study, all of which yielded null results. The major differences in procedures between the preceding and current experiments are summarized in

Table S4.4. Based on the null findings of preceding experiments, we improved the design of the current study so that (1) visual stimuli were presented for a longer period (12 s), (2) the number of trials was reduced, (3) two individuals' vocalizations were played alternately within a trial. Note that we conducted Experiment 2 with a different group of chimpanzees to ensure that the findings from Experiment 1 were not by-products of repeated testing on the same participants.

Table S4.4 Summary of task features across experiments.

Experiment	Image pair	Test playback	Control playback	Stimulus duration and onset
Main text	Snake vs. Fruit	Alarm call, Food grunt, Scream	No sound	12-s playback simultaneous to 12-s movie
S1	Food vs. Non-food object	Food grunt	Pant hoot	6-s playback preceded 3-s static image by 3 s
S2	Food vs. Non-food object	Food grunt	Pant hoot	10-s static image preceded 6-s playback by 4 s
S3	Snake vs. Butterfly	Alarm call	Food grunt	6-s playback preceded 3-s movie by 3 s
S4	Snake vs. Food	Alarm call, Food grunt	-	6-s playback preceded 3-s movie by 3 s

Note. The six chimpanzees at Kumamoto Sanctuary participated in Experiments S1−4, and some of the chimpanzees at PRI participated in Experiment S1. Some of the auditory and visual stimuli were the same as those used in the experiments reported in the main text.

Supplementary References

Howell, D. C. (2010). *Statistical methods for psychology* (7th ed.) (pp. 461−513). Cengage Learning.

Chapter 5

General Discussion

5.1 Evolution of Understanding of Others: Differences Between Chimpanzee and Human Cognition

The current thesis presented three studies of chimpanzees, which examined their cognitive processes related to others in irregular states (injuries in *Chapter 2* and biomechanically impossible/possible body movements in *Chapter 3*) and others reacting to environmental disturbances (gaze responses to images of fruit/snake videos upon hearing playbacks of others' food grunts/alarm calls in *Chapter 4*). These experiments shed light on cognitive processing by using eye tracking methods to examine chimpanzees' gaze responses. In addition, I sought to investigate the affective aspects of these phenomena using contact-free physiological measurements (skin temperature measurement in *Chapter 2* and pupillometry in *Chapter 3*) and by testing an alternative hypothesis regarding affective responses (*Chapter 4*).

In *Chapter 2*, chimpanzees were found to look at images of injured conspecifics for longer than at images of non-injured individuals, suggesting that they discriminated injured from non-injured conspecifics and had more interest in the former. Moreover, a greater decrease in nasal skin temperature was observed when chimpanzees witnessed a human experimenter's injury, which involved an artificial open wound and running blood, suggesting that at least some individuals exhibited affective arousal upon seeing others' injury. However, chimpanzees were not found to exhibit affective reactions when observing injuries that involved a needle penetrating a fake thumb, which was a novel scenario for the chimpanzees.

In *Chapter 3*, chimpanzees did not clearly distinguish impossible from possible arm movements, suggesting that they may not have understood the structural properties of others' bodies (i.e., biomechanics). This result might also be related to the fact that nonhuman primates often seem to have difficulty processing visual displays of biological motion (Matsuno 2021). However, some chimpanzees exhibited signs that they distinguished impossible from possible arm movements displayed by an animated robot, suggesting that they may possess some knowledge about biomechanics, which the experiments were not able to uncover because of procedural factors (e.g., the uncanny nature of computer-generated animations). One study also obtained an ambiguous result as to whether chimpanzees detected irregular body part locations when they saw photos of chimpanzees with some body parts displaced (Gao et al. 2022). No clear difference in pupil dilation was exhibited between situations in which chimpanzees viewed possible and impossible movements, suggesting that they did not exhibit affective reactions when seeing others performing impossible body movements. However, it is likely that this lack of difference was also caused by procedural factors (e.g., the visual properties of the animation stimuli and the relatively small sample size).

In *Chapter 4*, chimpanzees were found to look at videos of snakes for longer when hearing alarm calls compared with when hearing food-associated calls or no sound. Moreover, playbacks of screams did not induce a gaze bias toward snakes, unlike alarm calls, suggesting that the effect of alarm calls may not solely be based on affective matching of negative arousal via vocalizations. Taken together, these results suggested that chimpanzees may have an association between alarm calls and images of snakes. However, chimpanzees were not found to look at videos of fruit for longer when hearing food-associated calls compared with when hearing no sound. This might indicate that chimpanzees do not associate their food-associated calls with images of fruit. Nevertheless, a possible effect of food-associated calls on gaze may have been difficult to detect in that experiment, considering that the chimpanzees were already biased to look at fruit videos even in the absence of auditory playback.

Although the null results should be interpreted with caution, these findings highlight the importance of experience or familiarity with given situations in chimpanzee cognition. Specifically, chimpanzees exhibited distinct reactions toward others in irregular but familiar and/or ecologically relevant situations, whereas chimpanzees were not found to react strongly toward others in unfamiliar or novel situations. Injuries, especially those involving open wounds and blood, and conspecific alarm calls in the presence of snakes may well be familiar to participating chimpanzees, and thus their responses may have been facilitated by past experiences of those events in their daily lives. In contrast, injuries involving needle penetration and biomechanically impossible body movements were unfamiliar for the participant chimpanzees, and thus elicited little or no response from them. Experiences have been suggested to play a role in cognitive processes for others' bodies (Gao and Tomonaga 2020), others' actions (Emery and Clayton 2001; Kuroshima et al. 2014; Kuroshima et al. 2017; but see Buttelmann et al. 2013 for a null result in chimpanzees), and empathic responding (e.g., Atsak et al. 2011; Church 1959; de Waal and Preston 2017; Goumon and Špinka 2016; Luo et al. 2020; Sanders et al. 2013; Sato et al. 2015; Toyoshima et al. 2021; Watanabe and Ono 1986; Wechkin et al. 1964). The findings presented in the current thesis may suggest that chimpanzees' understanding of others is more strongly rooted to past experiences, whereas humans' understanding of others is also modulated by some cognitive faculties, such as elaborated simulative processes (Decety and Grèzes 2006; Gallese 2014; Myers and Hodges 2008), that possibly facilitate understanding others in unfamiliar situations. Similar reasoning was reported by Morita al. (2012) regarding the perception of biomechanical movements in human adults vs. infants (see *Chapter 2*).

In terms of methodology, most of the experiments in the current thesis examined chimpanzees' gaze responses to visual stimuli, which were presented on a monitor (i.e., photos, computer-generated animations, and videos), using eye tracking. However, this method tells us little about the extent to which chimpanzees were absorbed in or immersed themselves in watching the stimuli (Kano et al. 2021). For example, a previous study in humans directly compared infants' responses to live and video stimuli and confirmed that infants exhibited less sustained looking, reduced reaching and weaker affective reactions of fear and interest toward videos compared with the corresponding live presentations (Diener et al. 2008). Moreover, in humans, differences between video and live presentations have been reported in a study on processing of others' body movements, possibly because of the greater ecological validity and unpredictability of the real stimuli over the video stimuli (Järveläinen et al. 2001; see also Shimada and Hiraki 2006).

Although comparable studies on chimpanzees are currently missing, one previous study examined whether chimpanzees learned to solve a simple task by observing a video of a conspecific performing the task (i.e., sliding a small door to either the right or left to reach a food reward behind it) (Hopper et al. 2012). In their first attempt after watching the video, fewer individuals matched the behavior of the model to solve the task (i.e., sliding the door in the same direction) compared with individuals who observed a live model, which led the authors to propose that the reduced "social salience" of videos is one of the factors responsible for this result.

In the current thesis, the results reported in Supplementary Materials in *Chapter 2* indicated that chimpanzees did not exhibit affective reactions upon seeing images of injuries and injection shown on a monitor, although this may also be because of other factors (see Supplementary Materials in *Chapter 2* for details). In contrast, in *Chapter 2*, the live demonstration of injury elicited physiological responses in chimpanzees. Intriguingly, a previous study found that videos of yawning conspecifics elicited contagious yawning from chimpanzees, whereas live presentation of yawning by a human experimenter did not (Amaci et al. 2014). It is likely that chimpanzees and humans attend to two-dimensional representations in a different way, either quantitatively and/or qualitatively, possibly impacting the differences in understanding of others expressed in experimental settings between chimpanzees and humans. In *Chapter 3*, animations of impossible movements failed to elicit chimpanzees' gaze responses that were comparable to findings in humans (Morita et al. 2012). Nevertheless, on the basis of the finding in *Chapter 2*, I speculate that chimpanzees might show different reactions when viewing biomechanically impossible movements performed in a real-life setting. This possibility should be examined in future research.

5.2 Implications for Chimpanzee Psycho-Physiology

In *Chapter 2*, I measured chimpanzees' nasal skin temperature using a thermo-camera to examine their affective responses upon seeing others' injury. The results highlighted the advantages of the contact-free thermal imaging in chimpanzee research. At the same time, however, there were limitations regarding the implementation of this method depending on the particular setting, species, or individuals being tested.

First, in winter, it was cold outdoors, and chimpanzees' facial temperature was low when they entered the indoor room from the outside enclosure. Because the indoor room was kept warm (approximately 20 °C), the facial temperature gradually increased, then became stable after several minutes (see also Kano et al. 2016; Sato et al. 2018). If the stimulus presentation began while the nasal temperature was still rising, the possible effect of the stimulus (i.e., a decrease in temperature) may have been masked by the ongoing increase in nasal temperature. If such a persistent temperature change is inevitable, removing the trend from data during analysis (Kano et al. 2016; Sato et al. 2018) may be possible. However, this process can distort the temperature changes that are dependent on an event of interest (i.e., the stimulus presentation) and is preferable to avoid if possible. Instead, it may be better to wait for the skin temperature to stabilize before the experiment begins. However, this can be difficult if individuals are not sufficiently tolerant of being kept inside for an extended period of time. Therefore, it is necessary to consider the outside temperature and chimpanzees' temperament when deciding when to start the stimulus presentation.

Second, during recording, I allowed chimpanzees to sip juice from a custom-made juice dispenser, aiming to keep chimpanzees sitting in front of the mesh with their head oriented forward (Sato et al. 2018). However, consumption of juice was found to affect nasal temperature, leading to a consistent temperature decrease (see also Sato et al. 2018). Thus, for experiments involving the presentation of stimuli, it may be better to give small pieces of food to attract chimpanzees (as in Kano et al. 2016). Nevertheless, one benefit of using juice was that it did not require a human experimenter to stay near the chimpanzees.

In *Chapter 3*, I attempted to use pupillometry to infer chimpanzees' affective responses upon seeing others perform impossible movements, using a similar method to that reported in a previous study in humans (Morita et al. 2012). However, this attempt was unsuccessful because I was not able to control the effects of visual properties of stimuli. Informal testing on myself indicated that pupil diameter may vary depending on which part of the stimulus is fixated on. Thus, to use pupillometry to probe chimpanzees' affective reactions effectively, further refinements in the experimental protocols are needed.

A major difficulty arose from the trade-off between the necessities of rigid control for visual properties and those of attracting chimpanzees' spontaneous attention to stimuli. Thus, when the visual properties of stimuli are strictly controlled for (e.g., by reducing differences in color and contrast within an image), the stimuli can become boring for chimpanzees, which is expected to reduce the time spent looking at stimuli and may make it difficult to measure pupil diameter reliably.

The results reported in *Chapter 3* indicate that the simple replication of previous pupillometry studies in humans may be difficult in chimpanzees, as argued in a previous eye tracking study on apes that was based on previous studies in human infants (Kano et al. 2017). Kano et al. (2017) mentioned that it was necessary to tailor the video stimuli deliberately so that apes were sufficiently interested, while avoiding excessive complexity. They achieved these goals by including agonistic interactions and by depicting only one action at a time with pauses between actions. The importance of making interesting stimuli has been recognized in human infant studies using pupillometry (Hepach and Westermann 2016; Krüger et al. 2020). Nevertheless, dedicated effort is necessary to create appropriate stimuli specifically for nonhuman great apes, and this process should be rooted in researchers' experiences of observations and interactions with them (Kano et al. 2021). Moreover, an ingenious technique used in a previous study of chimpanzees (i.e., presenting a light to induce pupillary reflex: Hepach et al. 2021) may also be useful in this context, inducing a pupillary reflex by flashing a light to measure minimal pupil diameter. Future studies should consider the characteristics of pupillometry as well as chimpanzee psychology.

In *Chapter 4*, I examined the possible role of affective processes in the effect of playbacks on chimpanzees' gaze by playing back irrelevant, arousing vocalizations (i.e., screams emitted in agonistic contexts). Assessing arousal via physiological measurements is thought to be useful for better delineating the effects of arousal from valence in attentional bias tests for anxious states (Monk et al. 2018), and to examine whether emotional expression of others has a contagious effect on receivers (Nieuwburg et al. 2021). Future studies combining cross-modal preferential looking with physiological measurements (e.g., thermal imaging or possibly pupillometry) may be promising for directly revealing the quantitative relationships between emotional contagion and gaze biases. In one previous study, when infant vervet monkeys (*Cercopithecus aethiops*) were presented with pairs of videos depicting two types of rhesus monkey (*Macaca mulatta*) vocal signals with an audio of either one, contrary to intuition, vervet monkeys looked at non-matching faces for longer (Zangenehpour et al. 2009). That study analyzed pupil diameters upon a single longest look at either the matching- and non-matching videos, and found a greater pupil diameter for the former, which suggested that playbacks of vocalizations synergized the saliency of videos of matching facial expressions, causing monkeys' aversion to the matching videos.

Presentation of a single video at a time might be useful for examining the effect of congruency between auditory and visual stimuli on prolonged gaze (reviewed in Adachi 2009), and greater pupil dilation (Krüger et al. 2020) may also provide a promising option. A recent cross-sectional study including 1- to 6-year-old children and adult human participants examined pupil responses while participants looked at photos of animals coupled with either matching or non-matching vocalizations (e.g., a photo of a dog with the sound of a horse neighing) and observed a greater pupil dilation in response to incongruent audio-visual combinations, whereas a longer looking time was found only for 2-year-olds and adult humans.

Alternatively, the effect of playbacks on affective states and gaze bias could potentially be assessed by, for example, playing back calls while presenting a blank screen and subsequent pairs of videos. Such successive presentation might not only allow the use of pupillometry while controlling for confounds caused by the visual properties of stimuli but also the examination of whether vocalization evokes visual imagery more stringently, as testing of visual imagery of an object requires observation of a certain response associated with that object but in the absence of the visual input of that object (Adachi 2009; Suzuki 2018).

Even in humans, for which a wealth of physiological measurements has been taken, a single physiological index or even multiple physiological indexes cannot serve as a "fingerprint" to identify a specific emotional state (e.g., anger, happiness) (Siegel et al. 2018). Similarly, a physiological indicator of arousal, such as peripheral skin temperature or pupil dilation alone, cannot determine the detailed nature of chimpanzees' affective experiences. Moreover, as Berntson and Boysen (1990) pointed out for cardiac responses, possible differences between species in basic physiological functioning should be clarified in the future.

It is likely that chimpanzees and humans have evolved a different set of affective processes, which serve different functions geared toward the environmental challenges faced by each species (Adolphs and Andler 2018; Paul and Mendl 2018). Thus, chimpanzees might exhibit different affective responses upon witnessing others' injuries and impossible movements compared with those of humans. In humans, observations of painful situations or injuries can elicit both disgust and vicarious pain (Kupfer 2018; Shenhav and Mendes 2014; Steinkopf 2017), and those feelings might be labeled as disgust simply because a more appropriate term is often difficult to find (Kupfer 2018). Thus, the observed skin temperature decrease in response to others' injury in *Chapter 2* might be interpreted as a sign of personal distress associated with past memories (Brooker et al. in press; Adriaense et al. 2020), empathy, disgust, or a type of undifferentiated arousal that has not yet been labeled appropriately, or a mixture of these. Future studies are needed to better understand affective processes in chimpanzees.

5.3 Limitations

It should be noted that the current studies involved several limitations. The findings reported in this thesis should be interpreted in light of these limitations.

First, the experiments were conducted with a relatively limited range of participants, with most data obtained from six chimpanzees housed at Kumamoto Sanctuary. In addition to their basic demographic characteristics, such as age and sex, apes have their own unique life histories (Idani and Hirata 2006), including differences in prior experiences of experiments and long-lasting bonds with certain researchers and caretakers. Because the behaviors of interest can exhibit large individual differences, the unique life histories of these chimpanzees need to be taken into account when interpreting the current findings. At the same time, however, it is these characteristics of chimpanzees, in combination with the researchers and caretakers working with them, that provide opportunities to probe aspects of ape cognition that would otherwise be inaccessible. These conditions enabled reliable recording of chimpanzee responses, such as gaze, nasal temperature, and pupil diameter. Thus, the findings of previous studies provide insights that may also be instructive for future research on other populations of chimpanzees or nonhuman animals in general.

Second, it is often difficult to select appropriate statistical analysis because of aspects of experimental designs and small sample sizes, coupled with large inter-individual differences, missing values, skewed data distributions, and temporal autocorrelation in data. In future, more sophisticated approaches should be considered to deal with these issues. Nevertheless, in the current studies, I attempted to plot individual data in figures as well as report null findings in the main text or supplementary materials to provide a picture of the current research that is as unbiased as possible. Accumulation of both positive and negative findings are helpful for future research (Matosin et al. 2014; Paul and Mendl 2018).

5.4 Conclusion

I carried out three studies to investigate chimpanzees' cognitive processes and the roles played by affective processes pertaining to the understanding of others. The first study presented in the current thesis suggested that chimpanzees look at others' injuries spontaneously rather than avoiding them and that some individuals exhibit affective reactions upon seeing others' injuries, particularly those involving conspicuous cues. The second study suggested that chimpanzees may not clearly understand the biomechanics underlying others' body movements, although one of the experiments suggested that chimpanzees may infer the biomechanics of a novel robot, possibly on the basis of their knowledge of biomechanics. The third study suggested that chimpanzees spontaneously associated conspecific alarm calls and images of snakes.

Although the findings and implications obtained in the present studies involved several limitations, I believe that they shed light on unexplored aspects of understanding of others in chimpanzees and provide useful information for future great ape research. I attempted to use contact-free physiological measurements to assess affective responses in chimpanzees. These attempts will contribute to methodological advances for the investigation of affective mechanisms in nonhuman animals. Reporting these methods in detail will contribute to further advancements in this field of research, contributing to the research area of “affectivism” (Dukes et al. 2021) from the perspective of comparative psychology.

5.5 References

Adolphs R, Andler D (2018) Investigating emotions as functional states distinct from feelings. Emot Rev 10(3):191–201. https://doi.org/10.1177/1754073918765662

Adriaense JEC, Koski SE, Huber L, Lamm C (2020) Challenges in the comparative study of empathy and related phenomena in animals. Neurosci Biobehav Rev 112:62–82. https://doi.org/10.1016/j.neubiorev.2020.01.021

Amici F, Aureli F, Call J (2014) Response facilitation in the four great apes: is there a role for empathy? Primates 55(1):113–118. https://doi.org/10.1007/s10329-013-0375-1

Atsak P, Orre M, Bakker P, Cerliani L, Roozendaal B, Gazzola V, et al. (2011) Experience modulates vicarious freezing in rats: a model for empathy. PLoS ONE 6(7):e21855. https://doi.org/10.1371/journal.pone.0021855

Brooker JS, Webb CE, Clay Z (in press) Primate empathy: a flexible and multi-componential phenomenon. In: Schwartz BL, Beran MJ (eds) Primate cognition studies. Cambridge University Press, Cambridge.

Buttelmann D, Carpenter M, Call J, Tomasello M (2013) Chimpanzees, *Pan troglodytes*, recognize successful actions, but fail to imitate them. Anim Behav 86(4):755–761. https://doi.org/10.1016/j.anbehav.2013.07.015

Church RM (1959) Emotional reactions of rats to the pain of others. Journal Comp Physiol Psychol 52(2):132–134. https://doi.org/10.1037/h0043531

Decety J, Grèzes J (2006) The power of simulation: imagining one's own and other's behavior. Brain Res 1079(1):4–14. https://doi.org/10.1016/j.brainres.2005.12.115

Diener ML, Pierroutsakos SL, Troseth GL, Roberts A (2008) Video versus reality: infants' attention and affective responses to video and live presentations. Media Psychol 11(3):418–441. https://doi.org/10.1080/15213260802103003

Dukes D, Abrams K, Adolphs R, et al (2021) The rise of affectivism. Nat Hum Behav 5(7): 816–820. https://doi.org/10.1038/s41562-021-01130-8

Emery N, Clayton N (2001) Effects of experience and social context on prospective caching strategies by scrub jays. Nature 414(6862):443–446. https://doi.org/10.1038/35106560

Gallese V (2014) Bodily selves in relation: embodied simulation as second-person perspective on intersubjectivity. Phil Trans R Soc B 369(1644):20130177. http://dx.doi.org/10.1098/rstb.2013.0177

Gao J, Adachi I, Tomonaga M (2022) Chimpanzees (*Pan troglodytes*) detect strange body parts: an eye-tracking study. Anim Cogn. https://doi.org/10.1007/s10071-021-01593-2

Gao J, Tomonaga M (2020) How chimpanzees and children perceive other species' bodies: comparing the expert effect. Dev Sci 23(6):e12975. https://doi.org/10.1111/desc.12975

Goumon S, Špinka M (2016) Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. Anim Cogn 19(3):501–511. https://doi.org/10.1007/s10071-015-0950-5

Hepach R, Vaish A, Kano F, et al (2021) Chimpanzees' (*Pan troglodytes*) internal arousal remains elevated if they cannot themselves help a conspecific. J Comp Psychol 135(2):196–207. https://doi.org/10.1037/com0000255

Hepach R, Westermann G (2016) Pupillometry in infancy research, J Cogn Dev 17(3):359–377. https://doi.org/10.1080/15248372.2015.1135801

Hopper LM, Lambeth SP, Schapiro SJ (2012) An evaluation of the efficacy of video displays for use with chimpanzees (*Pan troglodytes*). Am J Primatol 74(5):442–449. https://doi.org/10.1002/ajp.22001

Idani G, Hirata S (2006) Studies at the Great Ape Research Institute. In: Washburn DA (ed) Primate perspectives on behavior and cognition. American Psychological Association, Washington, pp 29–36

Järveläinen J, Schürmann M, Avikainen S, Hari R (2001) Stronger reactivity of the human primary motor cortex during observation of live rather than video motor acts. Neuroreport 12(16):3493–3495.

Kano F, Hirata S, Deschner T, et al (2016) Nasal temperature drop in response to a playback of conspecific fights in chimpanzees: a thermo-imaging study. Physiol Behav 155:83–94. https://doi.org/10.1016/j.physbeh.2015.11.029

Kano F, Krupenye C, Hirata S, Call J (2017) Eye tracking uncovered great apes' ability to anticipate that other individuals will act according to false beliefs. Commun Integr Biol 10(2):e1299836. https://doi.org/10.1080/19420889.2017.1299836

Kano F, Sato Y, Yamanashi Y (2021) How chimpanzees look at movies: the "Art and Science" project in Kyoto City Zoo. Japanese J Anim Psychol 71(1):13–26. https://doi.org/10.2502/janip.71.1.1

Kuroshima H, Kaiser I, Fragaszy DM (2014). Does own experience affect perception of others' actions in capuchin monkeys (*Cebus apella*)? Anim Cogn 17(6):1269–1279. https://doi.org/10.1007/s10071-014-0760-1

Kuroshima H, Nabeoka Y, Hori Y, Chijiiwa H, Fujita K (2017). Experience matters: dogs (*Canis familiaris*) infer physical properties of objects from movement clues. Behav Processes 136:54–58. https://doi.org/10.1016/j.beproc.2017.01.013

Krüger M, Bartels W, Krist H (2020) Illuminating the dark ages: pupil dilation as a measure of expectancy violation across the life span. Child Dev 91(6):2221–2236. https://doi.org/10.1111/cdev.13354

Kupfer TR (2018) Why are injuries disgusting? Comparing pathogen avoidance and empathy accounts. Emotion 18(7):959–970. https://doi.org/10.1037/emo0000395

Luo WJ, Li CL, Geng KW, Wang XL, Du R, Yu Y, et al. (2020). The similar past pain experience evokes both observational contagious pain and consolation in stranger rat observers. Neurosci Lett 722:134840. https://doi.org/10.1016/j.neulet.2020.134840

Matosin N, Frank E, Engel M, Lum JS, Newell KA (2014) Negativity towards negative results: a discussion of the disconnect between scientific worth and scientific culture. Dis Model Mech 7(2):171–173. https://doi.org/10.1242/dmm.015123

Matsuno T (2021) Visual body perception in primates: from individual to social dyad. In: Anderson JR, Kuroshima H (eds) Comparative cognition. Springer, Singapore, pp 83–103. https://doi.org/10.1007/978-981-16-2028-7_6

Monk JE, Doyle RE, Colditz IG, et al (2018) Towards a more practical attention bias test to assess affective state in sheep. PLoS ONE 13(1):e0190404. https://doi.org/10.1371/journal.pone.0190404

Morita T, Slaughter V, Katayama N, et al (2012) Infant and adult perceptions of possible and impossible body movements: an eye-tracking study. J Exp Child Psychol 113(3):401–414. https://doi.org/10.1016/j.jecp.2012.07.003

Myers MW, Hodges SD (2008) Making it up and making do: simulation, imagination, and empathic accuracy. In: Markman KD, Klein WMP, Suhr JA (eds) Handbook of imagination and mental simulation. Psychology Press, New York, pp 281–294

Nieuwburg EGI, Ploeger A, Kret ME (2021) Emotion recognition in nonhuman primates: How experimental research can contribute to a better understanding of underlying mechanisms. Neurosci Biobehav Rev 123:24–47. https://doi.org/10.1016/j.neubiorev.2020.11.029

Paul ES, Mendl MT (2018) Animal emotion: descriptive and prescriptive definitions and their implications for a comparative perspective. Appl Anim Behav Sci 205:202–209. https://doi.org/10.1016/j.applanim.2018.01.008

Sanders J, Mayford M, Jeste D (2013) Empathic fear responses in mice are triggered by recognition of a shared experience. PLoS ONE 8(9):e74609. https://doi.org/10.1371/journal.pone.0074609

Sato N, Tan L, Tate K, Okada M (2015) Rats demonstrate helping behavior toward a soaked conspecific. Anim Cogn 18(5):1039–1047. https://doi.org/10.1007/s10071-015-0872-2

Sato Y, Kano F, Hirata S (2018) Cutting-edge infrared thermography as a new tool to explore animal emotions. Japanese J Anim Psychol 68(1):1–15. https://doi.org/10.2502/janip.68.1.7

Shenhav A, Mendes WB (2014) Aiming for the stomach and hitting the heart: dissociable triggers and sources for disgust reactions. Emotion 14(2):301–309. https://doi.org/10.1037/a0034644

Shimada S, Hiraki K (2006) Infant’s brain responses to live and televised action. Neuroimage 32(2):930–939. https://doi.org/10.1016/j.neuroimage.2006.03.044

Siegel EH, Sands MK, Van den Noortgate W, et al (2018) Emotion fingerprints or emotion populations? A meta-analytic investigation of autonomic features of emotion categories. Psychol Bull 144(4):343–393. https://doi.org/10.1037/bul0000128

Steinkopf L (2017) Disgust, empathy, and care of the sick: an evolutionary perspective. Evol Psychol Sci 3(2):149–158. https://doi.org/10.1007/s40806-016-0078-0

Toyoshima M, Mitsui K, Yamada K (2021) Prior stress experience modulates social preference for stressed conspecifics in male rats. Neurosci Lett 765:136253. https://doi.org/10.1016/j.neulet.2021.136253

Watanabe S, Ono K (1986) An experimental analysis of “empathic” response: effects of pain reactions of pigeon upon other pigeon's operant behavior. Behav Processes 13(3):269–277. https://doi.org/10.1016/0376-6357(86)90089-6

Wechkin S, Masserman JH, Terris W (1964) Shock to a conspecific as an aversive stimulus. Psychon Sci 1(2):47–48. https://doi.org/10.3758/BF03342783

Zangenehpour S, Ghazanfar AA, Lewkowicz DJ, Zatorre RJ (2009) Heterochrony and cross-species intersensory matching by infant vervet monkeys. PLoS ONE 4(1):e4302. https://doi.org/10.1371/journal.pone.0004302

Acknowledgments

In addition to the acknowledgments provided for each study, I thank Dr. Hirata, my supervisor, for providing thoughtful support and valuable teaching, and for encouraging my research. I also thank Dr. Morimura for his cooperation in this research and valuable teaching about chimpanzees. I am grateful to Dr. Kano for generously sharing technical know-how, including experimental designs for apes and writing papers. I give special thanks to current and previous staff at Kumamoto Sanctuary for their dedicated care of apes, as well as enabling, encouraging, and helping my research, and for their kind administrative support for trips and purchasing equipment. I am thankful for the opportunity to engage in great ape research in this environment.

I thank the members of the Wildlife Research Center, Kyoto University, and Primatology and Wildlife Science, Leading Graduate Program at Kyoto University, for providing me with valuable lessons and opportunities, and for their kind administrative supports. I thank Benjamin Knight, MSc., from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript. Finally, I am grateful to the bonobos and chimpanzees involved in this research, including the six chimpanzees who participated in all of my research (Hatsuka, Iroha, Misaki, Mizuki, Natsuki, and Zamba), for providing a view into their intriguing minds. I hope that each of them will live a healthy life.