Abstract

Facial Expressions and Behaviours Associated with Pain in Japanese Macaques

(ニホンザルにおける痛みに関連した表情および行動に関する研究)

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Chapter 1. General Introduction

Nonhuman primates (NHP) are extensively used in biomedical research due to their close evolutionary relationship with humans. Invasive research might cause pain, affecting the animals' quality of life and biasing study outcomes. Pain is a complex, unique experience with sensory, emotional and cognitive components; thus significant interindividual variability in pain expression is expected. Pain assessment in NHP is challenging because macaques tend to mask pain when an observer is present. Moreover, few signs of pain have been documented in NHP species, making it harder to know where observers should focus their attention when evaluating animals. Several methods can be used to assess pain in macaques, including physiological variables, behaviour, vocalisation and facial expressions. Automated systems have recently been implemented for pain recognition in animals due to their potential to help reduce animal suffering while reducing the time of human annotation bias and fatigue-related errors.

The main aim of this thesis was to identify pain indicators in captive Japanese macaques. The female macaques observed in this study underwent laparotomy for other research purposes, and data were collected opportunistically. Analgesics were administered during surgery and for three days after surgery. The hypotheses were that 1) animals decrease their overall activity after surgery due to pain or discomfort, 2) animals would show facial signs associated with pain, mainly one day after surgery, but not one week later, and 3) automated identification of faces from videos recordings followed by pain classification using deep learning would have accuracy above chance.

Chapter 2. Assessment of postoperative behaviour in captive Japanese macaques

Chapter two explores the differences between preoperative and postoperative behaviour in captive macaques. For that, an ethogram was developed to code the amount of time spent in postures (bipedal walk, quadrupedal walk, lying, sitting, standing on two feet, standing on four feet, and praying position) and activities (licking, playing, pulling hair, self-grooming, and touching the wound). Behaviours were compared in three conditions: preoperative period as the nonpainful

baseline, one day after surgery before receiving analgesics (considered the most painful time during the study period), and after analgesia (expected to be less painful). General linear mixed-effect models were used to test predictions about the influence of condition and time of day (AM/PM) on the proportion of time spent doing an activity or a given posture. Analysis showed that the proportion of time that macaques displayed postures and activities did not significantly change between conditions. However, one individual spent more time in the praying position before receiving analgesia than the baseline. In addition, more playing time and self-grooming were observed during baseline and after receiving analgesia. The results suggest that pain assessment and treatment strategies should be discussed case-by-case for macaques in captivity.

Chapter 3. Investigating subtle changes in facial expression to assess acute pain in Japanese macaques

Facial expressions are a source of nonverbal communication in many mammalian species. This study used geometric morphometrics to explore the facial shape variation in female Japanese macaques who underwent laparotomy. Face image samples were collected from video footage of fourteen macaques before surgery and 1, 3, and 7 days after the procedure. Image samples in the pre-surgical condition were considered pain-free, and facial expressions emerging after surgery were investigated as potential indicators of pain. Landmarks for shape analysis were selected based on the underlying facial musculature and their corresponding facial action units and then annotated in 324 pre-surgical and 750 post-surgical images. The results show that expressions of pain are likely to vary between individuals. Tightly closed eyelids or squeezed eyes and lip tension were the most common facial changes one day after surgery ($p < 0.01974$). Geometric morphometrics was a valuable tool for evaluating subtle changes in the facial expressions of Japanese macaques and provided a better understanding of facial cues to pain for captive macaque care.

Chapter 4. Macaque face detection to assess pain using deep learning

Artificial Neural Networks became a feasible option for image classification tasks. Twenty-two Japanese macaques were recorded undisturbed in their cages before (No Pain) and one day after laparotomy before scheduled analgesia (Pain). Videos were processed for facial localisation using RetinaFace or Mask-RCNN algorithms. RetinaFace extracts a squared picture of the face (Box extraction), while Mask-RCNN extracts the contour of the face (Contour extraction). ResNet50

was used for the classification of the pictures. Seventy-five per cent of the images were labelled (Pain or No Pain) and used for training the neural network. Images used for testing (25%) were not in the training data. After Box extraction, test accuracy results were 0.47 to 0.48. The Box extraction followed by classification using ResNet50 model presented low accuracy for generalisation to any given macaque, likely due to memorisations of features irrelevant for pain: background, brightness, skin colour, or objects in the enclosure. Contour extraction was then used, and pictures were pre-processed for normalising colour and brightness. The results obtained with Mask-RCNN followed by pre-processing and classification using ResNet50 improved to 63% ± 2. Results suggest that Mask-RCNN is suitable for extracting facial features from videos and that the performance of the classifying model is satisfactory for single-frame images.

Chapter 5. General Discussion

The results suggest eye tightening, asymmetrical aperture of the eye, lip tension and behavioural changes after laparotomy are potentially associated with pain. After surgery, the individuals might simultaneously experience pain, anxiety, fear, anger, and nausea. Untangling these emotions is not feasible, and it is an unavoidable limitation of studying pain in clinical settings. However, given the context (surgeries cause pain), the analogy to painful procedures in humans (laparotomy and uterine manipulation are painful according to self-reports from women), the neuroanatomical structures of primates and the current understanding of pain in animals, it is likely that pain is happening after laparotomy in macaques. The use of analgesics reduced pain intensity in the postoperative period. Buprenorphine is an opioid analgesic commonly used in NHP, and carprofen is a nonsteroidal anti-inflammatory drug used to control postoperative pain and inflammation following surgery. Regardless, the laparotomy model allowed insights into actual pain-related clinical conditions. This work also demonstrated that machine learning could classify pain images without a priori annotation.

The behavioural and facial expression changes indicate that captive Japanese macaques present signs potentially associated with pain. It also emphasises the importance of individualised assessment and provides information that could be used to develop practical, non-invasive scales for assessing pain in macaques. A better understanding of the pain signs in captive macaques will ultimately lead us to improve their overall care.