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研究課題名（和文）Geometric morphometrics for the study of facial expression of pain in common marmosets

研究課題名（英文）Geometric morphometrics for the study of facial expression of pain in common marmosets

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研究成果の概要（和文）：この研究の目的は、コモンマーモセットの疼痛に関連する表情を明らかにすることであった。幾何学的形態測定法（GMM）を用いて、他の研究目的の開腹手術の機会に、術前後の顔の形状変化を評価した。GMM分析により、目や口のまわりの緊張や、耳の房毛の上昇など、術後の痛みに関連する可能性のある顔の変化が明らかになった。疼痛に関連する同様の顔の変化は、最近ニホンザルでも確認されている。この研究により、GMMは非ヒト霊長類の疼痛を評価するための有用なツールであり、疼痛の認識と福祉改善という目標に貢献することが示唆された。このプロジェクトの資金援助により、2つの関連論文が出版され、3番目の論文が準備中である。

研究成果の学術的意義や社会的意義
This study identified face shape changes in common marmosets potentially associated with pain using geometric morphometrics. This contributes towards better pain recognition, which will ultimately lead to improved data reliability and validity in marmoset studies aimed at improving human health.

研究成果の概要（英文）：The aim of this study was to identify facial expressions associated with pain in common marmosets. We used geometric morphometrics (GMM) to evaluate face shape changes after routine laparotomy (abdominal) surgery. The GMM analysis revealed face shape changes potentially associated with postoperative pain, including contraction of the eye and mouth areas and raising of the ear tufts. Recently, similar face shape changes associated with pain have also been identified in Japanese macaques. This study provides further evidence that GMM is a useful tool for assessing pain in non-human primates, which contributes towards the overall goal of improving pain recognition and welfare. The funding for this project supported the publication of two related papers and a third paper is in preparation.

研究分野：Laboratory Animal Science

キーワード：geometric morphometrics　facial expression　pain　behaviour　common marmoset

１．研究開始当初の背景
Background: The common marmoset is an increasingly popular animal model of human health and disease [1]. Despite this research involving potentially painful procedures, there are currently no standardized methods for assessing pain in marmosets. In biomedical research it is critical that pain is minimized or prevented because data reliability and validity may be compromised when taken from animals in pain [2]. In the past decade, tools have been developed to identify facial expressions associated with pain in mice [3], rats [4], rabbits [5], sheep [6] and horses [7]. These are based on facial 'Action Units' used to quantify change in muscle movement derived from the human Facial Action Coding System (FACS) [8]. In this project we developed CalliFACS [9, 10]; a new anatomical tool to objectively describe facial musculature and movement specific to common marmosets. We then developed a set of 48 landmarks based on CalliFACS to quantify how face shape varies with increasing pain intensity.

Morphometrics refers to the quantitative analysis of form, including size and shape. Geometric morphometrics (GMM) is a morphometric method to analyze and compare the whole landmark configuration of an object instead of just using single measurements. It is used to study how shapes vary as well as their covariance with other variables. Recently, GMM has been used to evaluate face shape changes in relation to increasing pain intensity in cats [11] and Japanese macaques [12] using facial landmarks. The advantages of GMM are that (1) it allows dynamic face shape variation to be visually mapped, and (2) it facilitates an objective, species-specific representation of how face shape changes at different time points in a procedure.

２．研究の目的
Purpose: The project had two main aims. First, to quantify changes in facial expressions of marmosets in relation to a painful procedure a using a GMM approach, and second, to validate these changes against traditional pain-related behaviours. The overall goal is to minimize or eliminate pain and subsequently improve the quality of biomedical research using common marmosets.

３．研究の方法
Methods:
Stage 1 - Data collection: The study was observational and carried out at the Central Institute for Experimental Animals (CIEA) in Japan. For ethical reasons pain was not deliberately induced for the purpose of our research. Data collection involved opportunistic sampling of laparotomies (abdominal surgery) conducted for other scientific purposes. Video was taken for 60 mins at three time points (TPs) (**Figure 1**); TP1: Pre-surgery (one-day before), TP2: Pre-analgesia (one-day post-surgery, before administration of additional analgesics) and TP3: Post-analgesia (one-hour after post-operative analgesia). An ethogram was developed to record the frequency of traditional pain-related behaviours to score pain intensity at each TP.

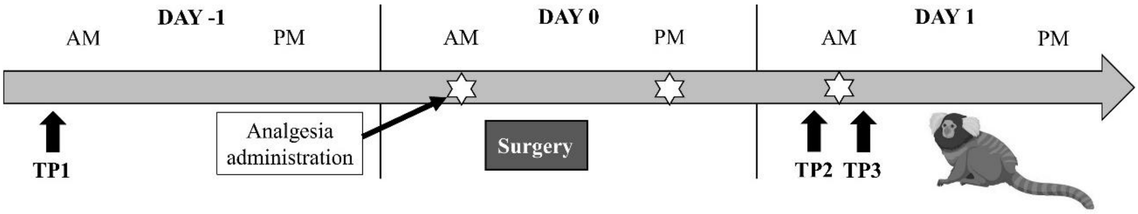

Figure 1. Timeline of procedure and time points (TPs) for video recording

Stage 2 - Geometric morphometrics: Image preparation: Images were extracted from video footage of common marmosets across the different time points (TP1-TP3) and manually annotated with 48 landmarks based on CalliFACS [9].

Data analysis: Extracted landmark data has a lot of variation in position, orientation and scale between images. These non-shape variations must be removed before further analysis. Shape information was extracted from the landmark coordinates with Generalized Procrustes fit. Landmark configurations were superimposed by translating, rotating and scaling all configurations to a common reference system (the mean). The resulting multidimensional geometric information was summarized using a Principal Component Analysis (PCA). To further explore group differences, we used Canonical Variate Analysis (CVA) to visualize the shape features that best distinguish between the three time points. In line with findings in cats [11] and Japanese macaques [12], the greatest face shape variation was expected to be present when pain intensity was assumed to be highest (TP2).

４．研究成果

Results: 528 facial images across the three time points (TP1: 235 images, TP2: 142 images, TP3: 151 images) were extracted from 21 marmosets who underwent laparotomy surgery.

Group analysis: Face shape changes were subtle in appearance and challenging to detect by direct visual inspection alone. The most obvious differences between pre-surgery (TP1) and post-surgery (TP2, TP3) were observed in the marmosets at TP2 (one-day post-surgery and before administration of additional analgesics). Face shape differences related to TPs were located using discriminate analysis. Face shape differences between TPs are presented in a Canonical Variate Analysis (CVA) scatterplot below (**Figure 2**). Along canonical variate 1 (CV1), TP2 and TP3 faces presented more eye and mouth contraction and raised ear tufts compared to TP1. Along canonical variate 2 (CV2), TP2 faces showed more eye contraction than TP1 and TP3 (although less pronounced than for CV1). Mahalanobis distances (a measure of the distance between a point and a distribution) among the three conditions were significantly different in all pairwise comparisons ($p < 0.05$), while Procrustes distances diverged ($p < 0.05$) (**Table 1**).

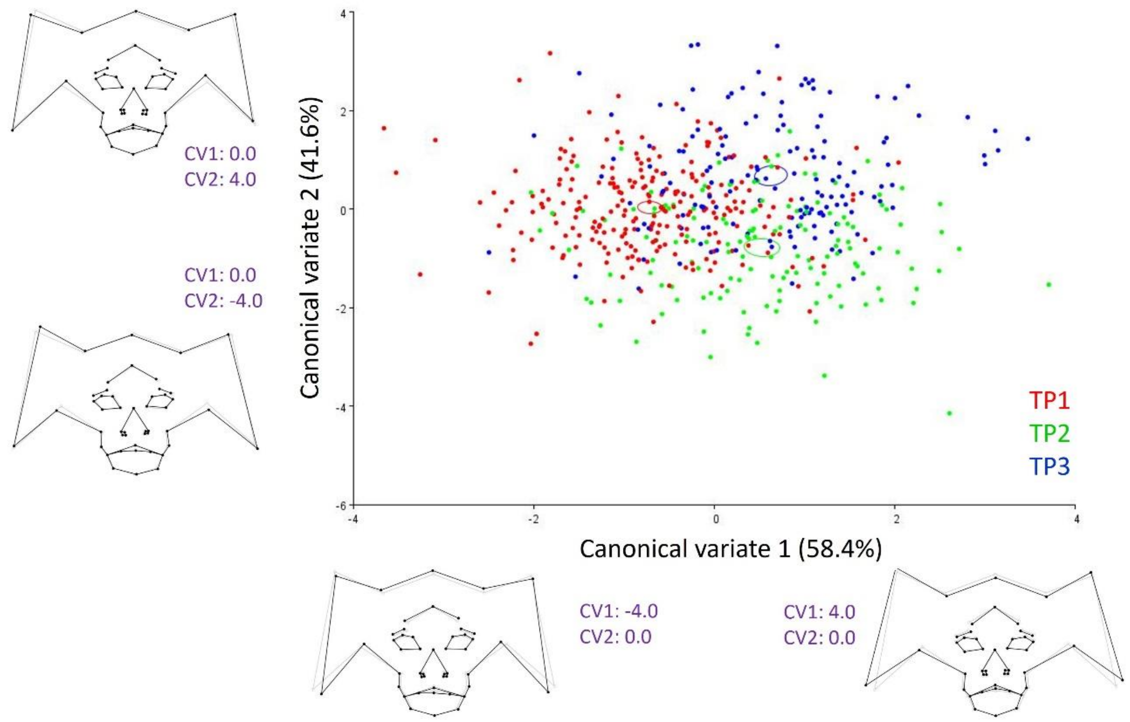

Figure 2. Face shape variation between time points (TPs) after Canonical Variate Analysis. Each colour refers to a TP, and confidence ellipses for means are given with a probability of 0.9. The face wireframes depict changes in shape along the two main canonical variates. The black wires indicate variation per unit of within-subject variation (Mahalanobis distances) relative to the grey-mean shape. The amount of variation explained by each CV is shown in brackets.

	Mahalanobis distances among groups			Procrustes distances among groups		
	TP1	TP2	TP3	TP1	TP2	TP3
TP1	-	0.0003	< 0.0001	-	0.0127	< 0.0001
TP2	1.4904	-	< 0.0001	0.0202	-	< 0.0001
TP3	1.4920	1.4654	-	0.0179	0.0150	-

Table 1. Differences in facial shapes between pre-operative (TP1) and post-operative time points (TP2, TP3). Mahalanobis distances (left) and Procrustes distances (right): p-values (above) and distances among groups (below).

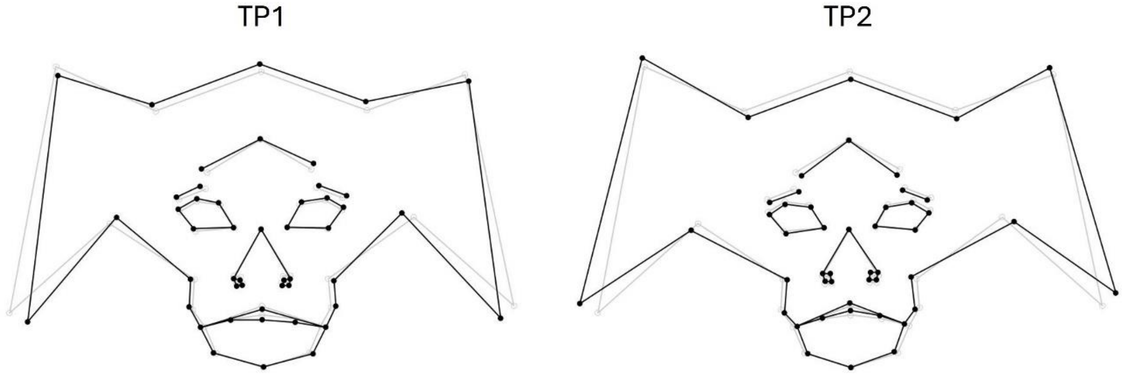

Figure 3. Geometric representation of all marmoset faces combined. Relative face shape changes are shown between TP1 (before surgery and pain free) and TP2 (one-day post-surgery and before administration of additional analgesics). In TP2, greater contraction of the eye and mouth area and raised ear tufts can be observed compared to TP1.

Discussion:

The aim of this study was to identify facial changes associated with post-operative pain in common marmosets. Expressions potentially associated with pain were observed; especially in the eyes, mouth and ear tuft areas. Overall, the marmosets presented contraction of the eyes and mouth and raising of the ear tufts. Contraction of the eyes and/or mouth has also been identified as a sign of pain in mice [3], rats [4], rabbits [5], sheep [6], horses [7], cats [11], Japanese macaques [12] and Cynomolgus macaques [13]. Cats in painful conditions present with contracted eyes and reduced distance between the cheeks, mouth and nose region [11]. Cynomolgus macaques experiencing pain show flinching of the facial muscles around the eyes and contraction of the skin at the top of the head [13]. Japanese macaques who had undergone laparotomy showed eye contraction and lip tension one-day after surgery [12]. In Japanese macaques, it was not possible to evaluate potential changes in the ear landmarks as they are covered by hair. In common marmosets, large tufts of white hair surrounding the ears are used for communication (e.g. flattened to signify submission, fear, and curiosity in new objects, or flicked to display dominance) [14]. Therefore, we also included landmarks around these tufts. Post-operatively, the tufts appeared more raised which may serve as another indictor of pain. Together, these studies in other animals provide external convergent validity of our findings.

There are several limitations of our study. First, we assume that facial changes observed after surgery are associated with pain. However, it is also possible that these expressions are associated with other emotional states such as fear and anxiety, which are beyond the scope of this study to investigate. In line with prediction, we observed that the most obvious face shape changes at TP2 (one-day post-surgery and before administration of additional analgesics) when pain with expected to be most intense. However, as we did not withhold anesthesia or analgesics for ethical reasons, some of the expressions we observed may be associated with their effects. As an alternative to observing a routine surgery, deliberately evoking non-clinical acute pain may help to stimulate a prototypical 'pain face', but this is less acceptable ethically. Lastly, we are in the process of completing the analysis of face shape changes for individual marmosets. Like in Japanese macaques [12], we expect to observe individual differences in the facial features involved in expressing pain. We are also analyzing the frequency of traditional pain-related behaviours at different time points. If we find more pain-related behaviours post-surgery this will help to validate the face shape changes we assume to be associated with pain.

In conclusion, GMM appears to be a useful tool for evaluating subtle changes in the facial expression of common marmosets. Face shape changes associated with pain include eye and mouth contraction and raised ear tufts. While some additional analyses is pending, GMM is a potentially useful addition to behaviour-based pain evaluation in common marmosets. This GMM study, along with others, contributes towards improving pain recognition and welfare in non-human primates used for biomedical research.

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５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Correia-Caeiro Catia、Burrows Anne、Wilson Duncan Andrew、Abdelrahman Abdelhady、Miyabe-Nishiwaki Takako	４．巻 17
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３．雑誌名 PLOS ONE	６．最初と最後の頁 e0266442
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pone.0266442	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Correia-Caeiro Catia、Mouri Keiko、Huffman Michael A.、Wilson Duncan A.、Wang Xitong、Miyabe-Nishiwaki Takako	４．巻 271
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３．雑誌名 Applied Animal Behaviour Science	６．最初と最後の頁 106177～106177
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オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関