Statistical analysis on characteristic whisker movements observed in reward processing

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***Abstract*—Internal states of the brain can be often reflected as facial expressions. However, how animals show their facial expression is largely unexplored. Here, we focus on mice and investigate whether their whisker movements could be a facial expression of their internal states related to reward processing. We trained three mice for an auditory association task and filmed their whiskers during the task performance after enough learning. We found that approximately 5-8 Hz periodic whisking was commonly observed during reward-associated *Go* cue presentation. Such whisking rarely occurred in *No-Go* cue trials or in *Go* cue trials where the mice were not motivated to get a reward. Furthermore, after acquiring a reward, the mice whisked with a more protracted set-point. Using machine learning, we could accurately indicate reward-anticipating and reward-acquiring trials only from whisker time plots. Our analyses suggest that mice exhibit stereotypic whisker movements as a part of orofacial movements related to reward anticipation and acquisition.**

I. Introduction

Humans often express their internal states as physical motions. In particular, orofacial activities are closely involved in physiological states and emotions [1], referred to as facial expressions. Facial expressions can be observed in other animals living through social interaction as well [2], [3]. For example, it is reported that mice show facial changes characterized by tightened eyes, flattened ears, nose swells and cheek swells in response to aversive stimuli such as emergence of intruders and cat odor [4]. On the contrary, their ear is apt to become pinker and wider in positive emotional state [5].

Whisker movements are a representative orofacial activity that enables rodents to localize and track objects in their environment [6], [7]. Besides such a sensorimotor control, it is recently reported that well-trained mice protract their whiskers in response to whisker stimulation associated with water reward in a classical conditioning task [8]. This implies that mice could express his emotion related to reward anticipation as whisker movements. However, whisker stimulation itself is known to induce reflective whisker movement through feed-forward sensorimotor signaling [9], [10]. Therefore, it is still controversial whether whisker movements could represent some aspects of internal states.

In order to investigate whisker movement irrelevant to whisker stimulation, we here developed an auditory association task where thirsty mice discriminate two different sound tones, only one of which is associated with water reward to be given after the sound cue presentation. By statistically analyzing whisker movements of the expert mice performing this task, we found characteristic whisker movements that could be an orofacial activity induced by anticipation and acquisition of water reward.

II. Materials and Methods

A. Experiments

The experiments were performed using three male C57BL6J mice (6-week-old or older), named KM1, KM2, and KM3, respectively. At least three days after implantation of a light-weight metal head-post, the mice started to be water-restricted. The mice were adapted to head restraint on the experimental setup through initial training to freely lick the water spout for receiving water reward (3-5 sessions, one session per day).

The mice were then trained to perform the following auditory association task. The goal of the task is to associate 3 kHz pure tone (2 s) with water availability within 1 s window after the offset of tone presentation. Each trial were started with the 3 kHz cue (Go cue) or the 15 kHz cue (No-Go cue) following a random inter-trial interval ranging from 3 to 9 s. If the mice licked in the 2 s preceding the time when the trial was supposed to occur, then the trial was aborted and a next trial started. Lick was detected with a piezo sensor attached to the water spout. After each training session, 1.0 – 1.5 g of wet food pellet was given to the mouse in order to keep its body weight more than 80% of the initial value. Behavioral control was carried out using a custom-written program on Python interfaced through Arduino Uno. All whiskers except for right C2 whisker were trimmed before experiments.

B. Trial categorization & Data Collection

Each trial in the training phase was classified into one of the following categories based on difference in presented cues and outcomes:

- **Hit** trial in which mice licked the water spout during the reward window following Go cue presentation, so that water reward was delivered. Here, the reward window was referred to as 1 s window after the offset of tone cue presentation.

- **Miss** trial in which mice did not lick during the reward window following Go cue presentation, so that water reward was not delivered.
- **False Alert (FA)** trial in which mice erroneously licked during the reward window following No-Go cue presentation, so that water reward was not delivered.
- **Correct Rejection (CR)** trial in which mice did not lick during the reward window following No-Go cue presentation, so that water reward was not delivered.

After each experimental day, learning performance of the mice was evaluated as two indices:

$$\text{Hit rate} = \frac{(\#\text{ Hit trials})}{(\#\text{ Hit trials}) + (\#\text{ Miss trials})}$$
$$\text{FA rate} = \frac{(\#\text{ FA trials})}{(\#\text{ FA trials}) + (\#\text{ CR trials})}.$$

The training phase continued until the performance indices reached more than 80% Hit rate and less than 20% FA rate.

Then, we started the recording phase to collect behavior data of the well-trained mice. In this phase, the whisker was filmed at 200 Hz with a high-speed camera (HAS-L1, Ditect) and behavioral data were acquired at a sampling rate of 2 kHz using a National Instruments board. The task is the same as in the training phase, except that reward delivery was omitted randomly in 10 % Go cue trials in some experimental days. Such omission trials were introduced to investigate characteristic reactions to acquisition of expected water reward. Due to the inclusion of omission trials, each trial in the recording phase was classified into one of the following six categories: the same four categories as in the training phase and

- **Omission Lick (OL)** trials in which mice licked during the reward window following Go cue presentation but water reward was not delivered.
- **Omission No-Lick (ON)** trials in which mice did not lick during the reward window following Go cue presentation though reward delivery was supposed to be omitted.

For convenience of data analysis, we also consider the following two super-categories:

- **Reward Anticipation (RA)** trials consisting of Hit and OL trials in which mice could anticipate water reward.
- **Non-RA** trials consisting of Miss, FA, CR and ON trials.

C. Data Analysis

After the experiments, whisker traces (time series of whisker angle) were extracted from the movies filmed by high-speed camera, using a whisker tracking plugin in Fiji (https://github.com/tarokiritani/WhiskerTracking). Here, the whisker angle was defined so that positive (and negative) change in the angle corresponded to protraction (and retraction) of the whisker. For each trial, the time-series data between 2 s before the onset of the auditory cue and 2 s after the offset of the reward window were retrieved then smoothed by applying a band-pass filter between 4 and 25 Hz for noise reduction.

By visual investigation of typical profiles in each trial category (Fig. 1), we observed some characteristic whisker movements: 1) In the RA trials, the whisker often showed large protraction in response to the auditory cue, followed by regular oscillation with small amplitude, but did not in the Non-RA trials; and 2) In the Hit trials, the set-point of whisking shifted to the direction of protraction during reward window, but did not in the OL trials.

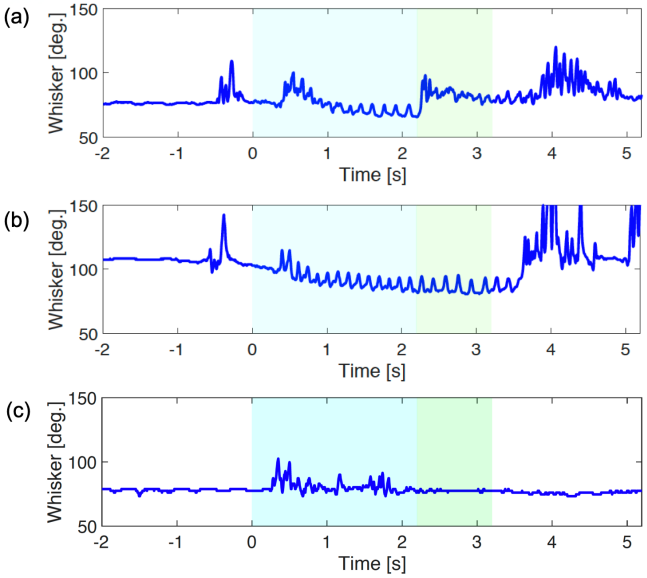

Fig. 1. Typical profiles of whisker movements observed in (a) a Hit trial, (b) an OL trial and (c) a CR trial. Different background colors show cue periods (blue), reward windows (green), and inter trial intervals (white).

To statistically examine those characteristics, we extracted two features from the time-series data for each trial:

- **Spatiotemporal pattern in cue period** was calculated as follows. Each time-series was transformed into a power spectrogram by short-time Fourier transformation with window size of 500 ms and overlapping 50%. The spectrogram only in region of interest (2 s cue period in time domain and 4-25 Hz in frequency domain) was extracted and normalized so that the sum of all frequency powers at each time should be one. We finally applied the principle component analysis to the normalized power spectrograms over all trials. The spatiotemporal pattern in cue period was defined as the first principal component score.
- **Spatiotemporal pattern in reward window** was the same as that in cue period, except that region of interest in time domain was 1 s reward window.
- **Shift of set-point in reward window** was defined as the median of the whisker angle during reward window substracted from that during cue period.

Then, the statistical difference in those features between trial categories was examined using Wilcoxon rank-sum test at significance level of $\alpha = 0.05$.

Assuming whisker movements were used for the mice to communicate their internal states relate to reward processing with each other, the observers could discriminate the states only based on the whisker movements with a high accuracy.

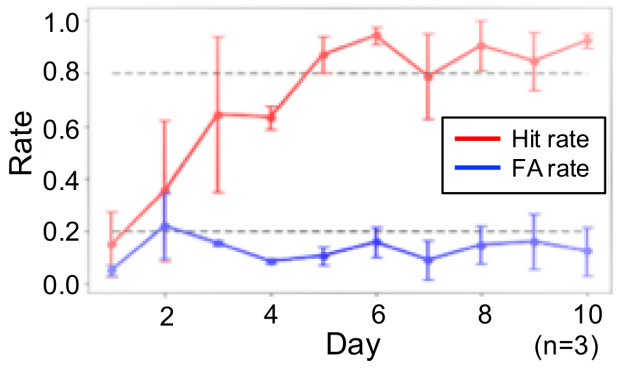

Fig. 2. Learning curves of the mice performing our auditory association task. Solid lines and error bars show means and their standard errors over three mice.

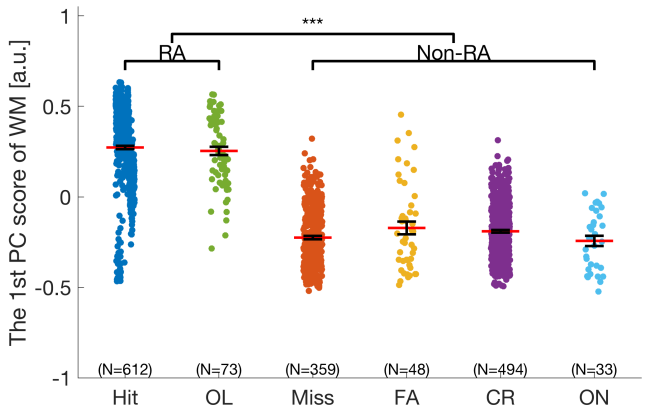

Fig. 3. Comparison of whisker movements in cue period among trial categories (*** $p < 0.001$, Wilcoxon rank-sum test). The spatiotemporal patterns were evaluated as the first principal component scores of whisker movement spectrograms. Red lines and error bars are means and their standard errors.

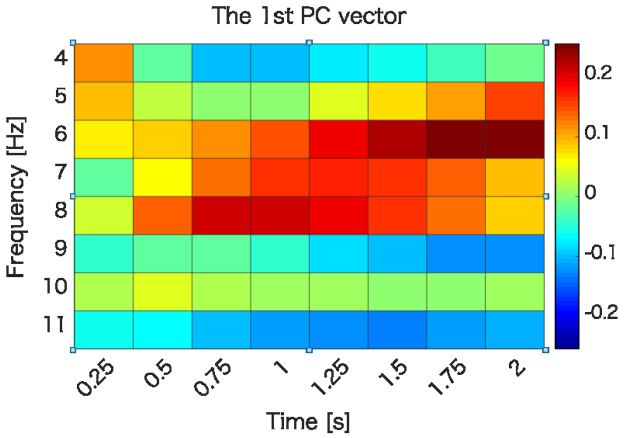

Fig. 4. Loading scores corresponding to spatiotemporal patterns in cue period (Fig. 3).

To investigate this possibility, we developed two models using machine learning technique. One was a logistic regression model trained to discriminate whether a spatiotemporal pattern in cue period corresponded to an RA trial or a Non-RA trial. The other was a linear discriminant analysis model trained to discriminate whether spatiotemporal pattern and shift of set-point in reward window corresponds to a Hit trial or an OL trial. The performance of the models were evaluated by leave-one-subject-out cross validation (i.e. the models were trained using data from two of three mice and tested using data from the remaining mouse.). Here, the confusion matrix, the accuracy, and the area under the receiver operating characteristic curve (AUC) was calculated for the performance indices.

III. Results

A. Training & Data Collection

Three mice sufficiently learned our auditory association task to achieve more than 80 % Hit rate and less than 20 % FA rate within 10 days after starting the training phase (Fig. 2). In the recording phase, KM1 performed 1049 trials for 7 days to collect time series data of whisker movements, while KM2 and KM3 performed only 220 and 350 trials, respectively, for 3 days due to technical reasons.

B. Whisker Movements in Cue Period

We merged whisker movement profiles of all mice together, and compared spatiotemporal patterns in cue period in the population level. The result showed significant difference between RA and Non-RA trials (Fig. 3). Also, large loading scores of the corresponding principal component were observed at 5-8 Hz in frequency domain of the power spectrogram (Fig. 4). Taken together, 5-8 Hz periodic whisker movements occurred during cue period in RA trials more frequently than Non-RA trials.

C. Whisker Movements in Reward Window

We then compared spatiotemporal patterns in reward window in the population level. There was significant difference between RA and Non-RA trials (Fig. 5(a)). On the other hand, there was no significant difference in between Hit and OL trials (Fig. 5(b)). Also, we compared shift of set-point in reward window. The result showed significant difference between RA and Non-RA trials (Fig. 5(c)). Taken together, 5-8 Hz periodic whisker movements continued until reward window in both Hit and OL trials, but the whisking in Hit trials shifted to a more protracted set-point.

D. Classification of Trial Categories

To investigate whether the observer could read out expectation for water reward from whisker movements in cue period, we trained a logistic regression model to classify whether a given spatiotemporal pattern in cue period was an RA trial or a Non-RA trial, then evaluated the classification performance by leave-one-subject-out cross validation. The result showed high performance with accuracy of 0.85-0.86 and AUC of 0.93-0.99 (Table I).

To investigate whether the observer could read out pleasure of water reward acquisition, we trained linear discriminant analysis model to classify whether a given pair of spatiotempo-

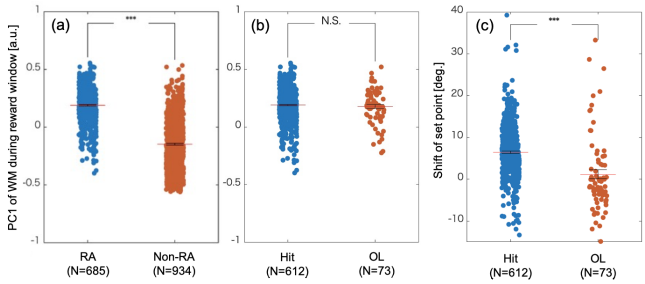

Fig. 5. Comparison of whisker movements in reward windows among trial categories (*** $p < 0.001$, Wilcoxon rank-sum test). (a and b) Comparison of spatiotemporal patterns in cue period (a) between RA and Non-RA trials, and (b) between Hit and OL trials. (c) Comparison of change in set-point in reward window between Hit and OL trials.

TABLE I
CLASSIFICATION PERFORMANCE OF WHISKER MOVEMENTS IN CUE PERIOD

			Prediction		Accuracy
			RA	Non-RA	(AUC)
Test Data	KM1	RA	375	37	0.85
		non-RA	118	519	(0.93)
	KM2	RA	71	0	0.85
		non-RA	21	128	(0.99)
	KM3	RA	138	3	0.86
		non-RA	34	86	(0.94)

ral pattern and set-point shift in reward window were a Hit trial or an OL trial, then evaluated its classification performance by leave-one-subject-out cross validation. The result showed high performance with accuracy of 0.89-0.93 and AUC of 0.71-0.93 (Table II).

IV. CONCLUSIONS AND DISCUSSIONS

In this study, we trained three mice for an auditory association task and analyzed their whisker movements in response to reward-associated tone and reward delivery. Our main findings were summarized as follows: 1) Approximately 5-8 Hz periodic whisking was commonly observed in Hit and OL trials, where the mice were expected to be highly motivated; 2) Such whisking rarely occurred in the other trials (i.e. Miss, FA, CR and ON trials), where the mice were not motivated to get a reward; and 3) The whisking shifted to a more protracted set-point after acquiring water reward. The results suggest that 5-8 Hz periodic whisking and protraction of the set-point could be a facial expression related to reward anticipation and acquisition, respectively.

We developed two classification models and showed that reward-anticipating and reward-acquiring trials could be accurately predicted only from information of whisker movements. The result implied that whisker movements could be used for the mice to communicate their internal states relate to reward processing with each other.

However, there are still open issues due to technical limitations. To get water reward in this task, mice have to take licking actions which could induce periodic whisking by orofacial muscle synergy. In addition, sniffing can happen in other reward anticipation tasks [11] and induce over 5 Hz periodic whisking [12]. Causality of such complicated interactions with multiple orofacial movements is important to elucidate neural mechanisms of facial expression related to reward processing.

TABLE II
CLASSIFICATION PERFORMANCE OF WHISKER MOVEMENTS IN REWARD WINDOW

			Prediction		Accuracy
			Hit	OL	(AUC)
Test Data	KM1	Hit	368	4	0.91
		OL	34	6	(0.71)
	KM2	Hit	61	2	0.93
		OL	3	5	(0.84)
	KM3	Hit	117	5	0.89
		OL	10	9	(0.93)

ACKNOWLEDGMENT

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REFERENCES

[1] P. Ekman, "Why lies fail and what behaviors betray a lie," in *Credibility Assessment*, J. C. Yuille, Ed. Dordrecht: Springer Netherlands, 1989, pp. 71–81.
[2] A. M. Burrows, B. M. Waller, L. A. Parr, and C. J. Bonar, "Muscles of facial expression in the chimpanzee (pan troglodytes): descriptive, comparative and phylogenetic contexts," *Journal of Anatomy*, vol. 208, no. 2, pp. 153–167, 2006.
[3] J. Kaminski, J. Hynds, P. Morris, and B. M. Waller, "Human attention affects facial expressions in domestic dogs," *Scientific Reports*, vol. 7, no. 1, p. 12914, 2017.
[4] E. B. Defensor, M. J. Corley, R. J. Blanchard, and D. C. Blanchard, "Facial expressions of mice in aggressive and fearful contexts," *Physiology & Behavior*, vol. 107, no. 5, pp. 680–685, 2012.
[5] K. Finlayson, J. F. Lampe, S. Hintze, H. Würbel, and L. Melotti, "Facial indicators of positive emotions in rats," *PLOS ONE*, vol. 11, no. 11, pp. 1–24, 2016.
[6] M. Deschênes, J. Moore, and D. Kleinfeld, "Sniffing and whisking in rodents," *Current Opinion in Neurobiology*, vol. 22, no. 2, pp. 243–250, 2012.
[7] L. E. McElvain, B. Friedman, H. J. Karten, K. Svoboda, F. Wang, M. Deschênes, and D. Kleinfeld, "Circuits in the rodent brainstem that control whisking in concert with other orofacial motor actions," *Neuroscience*, vol. 368, pp. 152–170, 2018.
[8] S. Sachidhanandam, V. Sreenivasan, A. Kyriakatos, Y. Kremer, and C. C. H. Petersen, "Membrane potential correlates of sensory perception in mouse barrel cortex," *Nature Neuroscience*, vol. 16, p. 1671, 2013.
[9] T. Yamashita, A. Pala, L. Pedrido, Y. Kremer, E. Welker, and C. C. H. Petersen, "Membrane potential dynamics of neocortical projection neurons driving target-specific signals," *Neuron*, vol. 80, no. 6, pp. 1477–1490, 2013.
[10] I. Ferezou, F. Haiss, L. J. Gentet, R. Aronoff, B. Weber, and C. C. H. Petersen, "Spatiotemporal dynamics of cortical sensorimotor integration in behaving mice," *Neuron*, vol. 56, no. 5, pp. 907–923, 2007.
[11] A. Kepecs, N. Uchida, and Z. F. Mainen, "The Sniff as a Unit of Olfactory Processing," *Chemical Senses*, vol. 31, no. 2, pp. 167–179, 2005.
[12] J. D. Moore, M. Deschênes, T. Furuta, D. Huber, M. C. Smear, M. Demers, and D. Kleinfeld, "Hierarchy of orofacial rhythms revealed through whisking and breathing," *Nature*, vol. 497, p. 205, 2013.