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学位論文題目	ゼブラフィッシュ幼魚における微細な姿勢制御の力学的・神経回路メカニズムの解明
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（様式３）

博士論文の要旨

氏　　名　　　相岡　拓己

論文題目

ゼブラフィッシュ幼魚における微細な姿勢制御の力学的・神経回路メカニズムの解明
(Biomechanics and neural circuit mechanisms of a fine postural control in larval zebrafish)

Postural control is important for survival in many animal species, regardless of whether they live on the land or in the water. Land-walking vertebrates maintain a desirable posture by a fine, static control, while they also recover from a severely disturbed posture by a vigorous, dynamic body movement. Although biomechanics of these postural control is well understood, neural mechanisms of the behaviors are not fully elucidated. When severely disturbed, fish recover their posture by vigorously performing swimming. Most fish also maintain their dorsal-up posture when they are still, which strongly suggests that fish finely control posture. However, the biomechanics and neural circuits of the fine postural control is unknown.

To examine fine control mechanisms in fish, I first observed larval zebrafish behavior from frontal and dorsal sides during roll-tilt stimuli in a dark condition. As previously reported, fish occasionally swam and recovered the upright posture. However, fish also recovered from the roll-tilted posture without swimming. The postural correction behavior remained in fish with pectoral fins removed. Upon close examinations, I found that fish slightly bent the body near the swim bladder to the ear-up side when they corrected their posture. The body bend reflex was more clearly observed when fish were placed in highly viscous water or head-embedded in agarose.

These results revealed that the fine postural correction is associated with a body bend behavior, which is referred to as vestibular-induced bend reflex (VBR).

How does the VBR recover the upright posture? In a simplified model, when a fish is upright, gravity, which acts at the center of mass (COM), and buoyancy, which acts at the center of volume (COV), are on the midline. In contrast, when a fish performs the VBR upon the roll tilt, the head and caudal body move toward the ear-up side, while in reaction, the body around the swim bladder moves toward the ear-down side. As the fish body bends, the COM position becomes more lateral than the COV position, because the density of the swim bladder is extremely smaller than that of the rest of the body. This results in a misalignment between gravity and buoyancy, generating a moment of force that counter-rotates the tilted body to the upright. If this model is correct, swim bladder-deflated fish would not be able to recover from the roll tilt when they perform the VBR, because the body density becomes nearly uniform, and therefore the misalignment between gravity and buoyancy is not produced. As expected, fish with deflated swim bladder were unable to recover. This result strongly supported the model. Thus, these results demonstrated that fish recover the upright posture by the fine postural control, which is the VBR.

Next, I focused on the neural circuits for the VBR. Based on the previous reports, I hypothesized that the VBR is driven by the following neural circuits: tangential nucleus (TAN; a vestibular nucleus) through neurons in the nucleus of the medial longitudinal fasciculus (nMLF; a class of RS neurons) to the spinal cord, and finally to the posterior hypaxial muscles (PHMs) near the swim bladder. To test this hypothesis, I performed two experiments for each cell population in the pathway: 1) Ca^{2+} imaging using a custom-built tiltable objective microscope and 2) behavioral analyses in head-embedded fish after cell ablation. During the roll tilt, TAN neurons in the ear-down side, a subset of nMLF neurons in the ear-up side, and slow-type PHMs in the ear-up side, especially those located in close proximity to the swim bladder, but not fast-type PHMs, were activated. As expected from the lateralized neural activity during the roll tilt, ablation of each cell population in the activated side impaired the VBR. These results revealed that the VBR is driven through the TAN-nMLF-PHM pathway. Taken together, the present study revealed biomechanics and neural circuits of a fine postural control in larval zebrafish.

Because fish are inherently unstable in the water, maintaining the dorsal-up posture requires neuro-muscular activity. Since most fish are upright almost all the time, fish likely maintain the dorsal-up posture by frequently performing the VBR.

Vestibulospinal neurons, which convey vestibular signals from the vestibular nucleus directly to the spinal cord, are thought to mainly mediate vestibular-induced postural responses. In addition to this pathway, it is presumed that pathways from the vestibular nuclei through reticulospinal neurons to the spinal cord are also involved in the postural control. Here the results clearly demonstrated that the reticulospinal neurons play an important role in the postural control in larval zebrafish. The present study suggests the importance of the reticulospinal pathways in other vertebrates and sheds light on the evolutionally conserved neural circuits involved in the postural control.

Form8・Separate Sheet
（様式８・別紙１）

Results of the doctoral thesis defense

博士論文審査結果

Name in Full
氏　　名　　　椙岡　拓己

Title
論文題目　　　ゼブラフィッシュ幼魚における微細な姿勢制御の力学的・神経回路メカニズムの解明

多くの動物にとって、姿勢制御は重要である。陸上生物の多くは、わずかな姿勢偏位時に、抗重力筋の活動を変化させるという微細な姿勢制御を行うことで、直立や背が上の状態に姿勢を維持している。この姿勢制御機構は、力学的にはよく理解されているが、神経回路メカニズムは完全には明らかとなっていなかった。一方、水中という全く異なる環境にいる魚においても、背を上にした状態を保持していることから、魚においても背を哺乳類に類似した微細な姿勢制御機構が存在する可能性がある。しかし、そのような機構が存在するかどうかはまったく不明であった。このような背景のもと、椙岡氏はまず微細な姿勢制御機構の有無を明らかすること、存在する場合、力学的、神経回路メカニズムの解明を目的に研究を行なった

椙岡氏は、まず微細な姿勢制御機構を調べるために、ロール方向への傾斜中のゼブラフィッシュの行動を観察した。その結果、傾斜姿勢からの立て直しに胴体の屈曲が重要であることを示した。力学的な観点から胴体の屈曲による姿勢制御の作用機構モデルを立てた。すなわち、胴体の屈曲はうきぶくろの相対的位置を横方向へ移動させ、これにより重力中心と浮力中心を横方向にずらすことで姿勢を立て直す方向への力のモーメントを生じさせ、魚は姿勢立て直している、というモデルである。そして、うきぶくろ内の気体を抜いた魚で行動実験を行うことにより、このモデルが正しいことを実証した。次に椙岡氏は、胴体の屈曲を司る神経回路の特定を試みた。神経回路の仮説を立て、カルシウムイメージングと細胞破壊した魚の行動実験を行うことで、屈曲に関わる神経回路を明確に示した。解明した神経回路のうち、網様体脊髄路ニューロンを介した経路は、これまで哺乳類で姿勢制御に関わると推測されていたものではあるが直接的な証拠が乏しい状態であった。今回の椙岡氏の研究は、網様体脊髄路ニューロンを介した経路が脊椎動物の姿勢制御に重要な役割を果たしていることをクリアに示したものである。

以上の研究から、椙岡氏はゼブラフィッシュにおいて微細な姿勢制御機構を明らかにし、その力学的メカニズムを非常に明確に示した。また、それを制御する神経回路を同定し網様体脊髄路ニューロンを介した経路の重要性を明瞭に示した、この結果は、水棲生物の動物行動分野に新たな知見をもたらすだけではなく、脊椎動物に共通する姿勢制御の神経回路メカニズムを考えるうえで重要な視点を与えるものと評価することができる。よって、本論文は同分野における重要な貢献であり、学位授与にふさわしいものであると審査委員全員が一致して結論した。