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Abstracts of Graduation Research Presentations, July 2023

July 7th 2023

College of Biological Sciences
University of Tsukuba

表紙画　Cover art

天野 史子　Amano Fumiko

表紙画の解説　Explanation of the cover art：

テーマは「切手」。モチーフは卒業生を対象に行なったアンケートの結果から選びました。

切手を貼れば日本中、世界中に手紙を届けることができます。筑波大学での学びや研究もまた、私たちを新たな世界へ連れて行ってくれることでしょう。

The theme is ″postage stamps″. The motif was chosen from the results of a survey conducted on graduates.

Stamps can be used to send letters to all over Japan and the world. The study and research at University of Tsukuba will also take us to a new world.

Graduation research presentation in Biology

生物学類卒業研究発表会（2023年度春）

July 7th, (11:30-12:40)

at 総合研究棟A110

-Program-

11:30–11:35
Greetings from English course faculty, Louis Irving John

11:35–11:50
Presenter: Park Kiwon
ASD-like phenotypes and brain abnormalities in *Usp15* KO mice

11:50–12:05
Presenter: Tawan Polsilapa
Optimisation of Glucose Concentration in Mixotrophic Cultivations of a Lichen Phycobiont *Elliptochloris subphaerica*

12:05–12:20
Presenter: Cerilles Tanisha Anne Sarmiento
Cold Stress-induced Oxidative Stress in Corals

12:20–12:35
Presenter: Lee Jennifer Jaime
Investigating the influence of positively valenced decorative visuals on knowledge gain and risk perception

12:35–12:40
Closing remarks from the Dean Kentaro Nakano

We look forward to your participation

どなたでもご参加いただけます。
皆様のご来場をお待ちしております。

Contact: gradtex@biol.tsukuba.ac.jp

ASD-like phenotypes and brain abnormalities in *Usp15* KO mice

Park Kiwon（筑波大学 生物学類）　　指導教員：鶴田文憲（筑波大学 生命環境系）

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disease defined by significant social, communication, and behavior challenges. Accumulating evidence suggests that genetic defect is the major cause of ASD. Ubiquitin carboxy-terminal hydrolase 15 (*Usp15*) is one of the candidate genes identified by the whole-exome sequencing of ASD patients. Previous studies have found that *Usp15* regulates autistic behavior and balance of excitatory/inhibitory (E/I) synapses. However, the mechanism of how the absence of *Usp15* leads to autistic behavior and abnormal brain structure remains unknown. In this study, I reported the differences in brain development and behavioral patterns between wild-type (WT) and *Usp15* knockout (KO) mice. I found that *Usp15* KO mice exhibit morphological abnormalities in the cerebral cortex and hippocampus, altered microglial state, and ASD-like behavior. These findings suggest that a defect in the *Usp15* gene is implicated in early brain development and the manifestation of behavioral patterns associated with ASD.

Material & Methods

1. Mice

Male and female WT and *Usp15* KO mice at 4-5 weeks of age were used for behavior experiments. *Usp15*$^{+/+}$, CX3CR1 GFP $^{+/-}$ mice and *Usp15* $^{-/-}$, CX3CR1 GFP $^{+/-}$ mice were generated by crossing *Usp15*$^{+/-}$ mice and CX3CR1 GFP $^{+/-}$ or CX3CR1 GFP $^{+/+}$ mice for microglial quantification. All mice used in this study were backcrossed with the C57BL/6J background.

2. Behavior tests

For the marble burying test, a total of twenty marbles were evenly arranged on 5cm-thick bedding in the plastic cage and 4-week-old mice were placed in the cage for 30 minutes. The number of marbles buried more than 75% were counted. For the nest-building test, the same size and weight of nesting material were placed in the cage with 4-week-old mice for 24h. Nests were scored based on the weight of intact nesting material and the shape of a nest.

3. Immunohistochemistry

The brains were perfused with PBS and fixed by 4% PFA/PBS overnight. After 30% sucrose infiltration, the samples were embedded in OTC compound and sliced at a 40-µm thickness. The brain sections were washed in PBS and permeabilized with 0.4% TritonX-100 in PBS, then blocked with 5% bovine serum albumin (BSA) and 0.4% Triton X-100 in PBS for 1 hour. Sections were incubated with primary antibodies diluted in a blocking solution at 4°C overnight. Sections were washed in PBS containing 0.4% TritonX-100 and incubated with secondary antibodies diluted in a blocking solution at room temperature for 2 hours. Fluorescence images were obtained using a fluorescence microscope (Leica Microsystems THUNDER 3D Cell Culture) and analyzed by FIJI ImageJ software.

Results & Discussion

Previously we identified that *Usp15* KO mice show autistic behavior by a three-chamber test. To assess detailed ASD-like behavior in *Usp15* KO mice, I performed additional behavior tests. In the marble burying test, *Usp15* KO mice buried significantly fewer marbles than WT mice. In the nest-building test, none of the *Usp15* KO mice could build a proper nest resulting in significantly lower nesting scores in *Usp15*KO mice compared to the WT mice. These results indicate that *Usp15*KO mice exhibit additional ASD-like behavior else than impaired sociability. Then I focused on the structural change of the brain from one of the most common autistic phenotypes, cortex thinning. Quantification of cortex thickness in the WT and *Usp15* KO mice at postnatal (P) 21 and P28 with the brain sections immunostained by DAPI showed that the cerebral cortex is significantly thinner in *Usp15*KO mice brains at P28.

Next, I focused on the hippocampus which is known to be highly involved in ASD. I found that *Usp15* KO mice exhibit abnormal dentate gyrus (DG) structures, which were significantly shorter in *Usp15*KO mice compared to WT at P28. This result is in line with previous studies that have reported abnormal DG development is involved in the expression of ASD-like behavior. These data demonstrate that a loss of the *Usp15* gene causes abnormal brain formation.

As it has been reported that microglia are associated with ASD and our finding supports the idea that impaired microglia are involved in the mechanism of ASD-like behavior and brain abnormalities. I therefore next quantified the number of microglia to identify potential molecular mechanisms. Quantification of the GFP signal at the cerebral cortex and hippocampus in the *Usp15*$^{+/+}$, CX3CR1 GFP$^{+/-}$ mice and *Usp15* $^{-/-}$, CX3CR1 GFP$^{+/-}$ mice at P28 showed that the density of microglia is significantly decreased in *Usp15*$^{-/-}$, CX3CR1 GFP$^{+/-}$. However, the expression level of Iba1, which is a marker of reactive microglia, was higher in *Usp15* KO mice. Thus, these results indicate that *Usp15* KO mice exhibit a decreased number of microglia in the cerebral cortex and hippocampus and impaired microglial states, which may contribute to imbalanced synapses and cortex thinning. Taken together, my results suggest that the absence of *Usp15* induces a decreased number of microglia and impaired microglial states that lead to ASD-like behavior and significant differences in brain morphology in *Usp15*KO mice.

Optimisation of Glucose Concentration in Mixotrophic Cultivations of a Lichen Phycobiont *Ellipitochloris subphaerica*

Tawan Polsilapa （筑波大学 生物学類）　　指導教員：Ishida Ken-Ichiro (筑波大学　生命環境系)

1. Introduction

The transition towards utilizing renewable resources for bioenergy has sparked considerable interest, with a particular focus on microalgae known for their high lipid content. Among microalgae, *Botryococcus braunii,* a trebouxiophycean green alga, is one of such microalgae and known for its high production of extracellular long-chain hydrocarbons, comprising up to 86% of its total dry weight (Borowitzka, 2018). However, *B. braunii* grow very slowly in culture, which raises the cost of hydrocarbon production.

Elliptochloris subsphaerica, a close relative of *B. braunii*, is a phycobiont of a lichen. In a previous study conducted at the laboratory to which I am affiliated, *E. subsphaerica* exhibited fast growth (3.5 g L^{-1} DCW in 14 days) and significant oil accumulation (30% of DCW) when cultured in medium X with 2% glucose (Shen, 2021). However, *E. subsphaerica* did not thrive in GTY medium, also containing 2% glucose (Shen, 2021), prompting questions about the role of glucose in its cultivation.

This experiment aims to identify the best glucose concentration for cultivating *E. subphaerica* with high biomass and lipid ratio. The effects of glucose concentrations in medium X ranging from 0% to 4% on growth characteristics and lipid accumulation were assessed.

2. Material & Methods

2.1. Culture media & Conditions: Five medium X with glucose concentrations of 0, 10, 20, 30, & 40 g L^{-1} each, along with a constant rate of other substances were prepared. Cells were inoculated at a concentration of $1x10^6$ cells per *mL,* under the light intensity at 250μ *mol* m^{-2} s^{-1} at 21°*C*, 100 rpm.

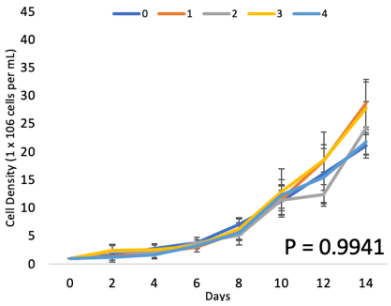
2.2. Cell Growth: The cellular growth was monitored biweekly, with observations taken once every two days via manual cell count by C-Chip (F01). Dry Cell Weight (DCW) was measured at the start and end of each experiment. Cells were harvested by centrifugation, froze at -80°*C*, and freeze-dried overnight.

2.3. Statistical Analysis: The analysis of data was carried out by Prism 9 software. The statistical significance was determined by analysis of variance (ANOVA). A P value < 0.05 was considered as statistically significant.

2.4. Observation of Lipid Accumulation: Cells collected on day 14 were stained with Nile-red dye at a ratio of 1:100 and observed under a fluorescent microscope.

3. Results & Discussion

3.1. Growth Characteristics: *E. subphaerica* did not present any significant differences in growth rate among the conditions, Fig 1 (p = 0.8912 - 0.9941). Mixotrophically cultured green algae such as *Chlorella pyrenoidosa* has been known to utilise monosaccharides such as glucose for their growth (Zhang el. al., 2014). On the contrary, my result indicates that *E. subphaerica* is not able to use glucose in mixotrophic condition but utilises alternative substances as its carbon source in the medium X, which differs from many previous reports. Moreover, *E. subsphaerica* achieved a high biomass yield (2.55 - 3.94 g L^{-1} DCW) and cell density (27.2 million cells mL^{-1}) over two weeks using a glucose-free medium. This suggests potential cost reduction in large-scale cultivation of *E. subsphaerica* for bioenergy, reducing the dependence on glucose.



3.2. Lipid Analysis:

Lipid droplets (LD) were observed in all conditions, with no significant difference (p > 0.9999) related to growth rate. Optimising LD accumulation and identifying unknown lipid constituents are crucial for utilizing *E. subphaerica* as a viable resource for oil production. If the significant portion of the unidentified accumulated lipid (approximately 80%) hydrocarbons (Shen, 2021), it would further enhance its potential as a valuable source for hydrocarbon production.

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Cold Stress-induced Oxidative Stress in Corals

Tanisha Cerilles（筑波大学 生物学類）　　指導教員：Sylvain Agostini（筑波大学 生命環境系）

Introduction:

Coral bleaching, characterized by the loss of symbiotic algae from corals, is primarily associated with elevated water temperatures, known as hot stress induced bleaching. However, cold water can also induce coral bleaching, impacting various physiological processes (Higuchi et al., 2015). Extensive research has focused on the mechanisms of coral bleaching under heat stress, highlighting the association between oxidative stress and subsequent bleaching events (DeSalvo et al., 2008). On the other hand, cold stress bleaching has been less studied compared to heat stress bleaching, resulting in a limited understanding of the mechanisms and impacts involved (Higuchi et al., 2015). Similar stress responses are observed in higher plants under cold stress to those reported for coral bleaching under hot stress, including disrupted photosynthesis and oxidative stress (Wang et al., 2017). The accumulation of reactive oxygen species (ROS) can cause damage to cellular components. These parallels between corals and higher plants suggest a common underlying mechanism for cold stress induced bleaching. The purpose of this study is to investigate whether oxidative stress occurs during cold-induced coral bleaching.

Lipid peroxidation (LPO) and total antioxidant capacity (TAC) assays were used to quantify oxidative stress in cold-stressed corals. Trial experiments confirmed reliable and reproducible quantification of LPO and TAC in the coral host.

Material & Methods:

A total of 12 microcolonies of *P. heronensis* were randomly selected. The microcolonies were then divided into two conditions: control condition(n=6) and cold stress(n=6), and then incubated for 48 hours.

In the control condition, the temperature was kept constant at 20° C throughout the entire 48-hour duration. In the cold stress group, the temperature was initially maintained at 20° C for 24 hours. Using chilling circulators, the temperature was gradually lowered from 20° C to 15° C over a span of 5 hours, with a decrease of 1° C per hour. The temperature was then maintained at a constant 15° C for an additional 24 hours.

Following the experimental period, a dark adaptation of 30 minutes was conducted on the microcolonies to measure the fluorescence (Fv/Fm). The physiological and biochemical performances of both the coral host and symbionts were measured.

Results:

Photosynthetic efficiency, lipid peroxidation levels, and total antioxidant capacity showed a significant difference between cold stress and control conditions (p= 0.0032, p= 0.03016, p= 0.04179, respectively). However, no significant results were observed for protein biomass, zooxanthellae density.

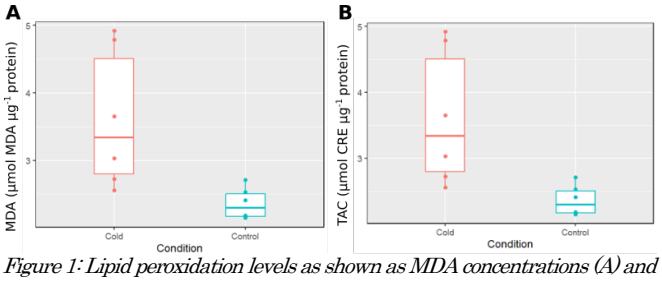

Figure 1: Lipid peroxidation levels as shown as MDA concentrations (A) and total antioxidant capacity (B) of corals exposed to short term cold stress.

Discussion:

Reduced photosystem efficiency is commonly observed in both hot stress-induced bleaching (Hoegh-Guldberg, 1999) and cold stress-induced bleaching (Roth et al., 2012).. The significant difference in total antioxidant capacity indicates compromised antioxidant enzyme generation, such as superoxide dismutase (Higuchi et al., 2015). This limited capacity contributed to ROS accumulation and increased lipid peroxidation levels, indicating oxidative damage. This imbalance in reactive oxygen species (ROS) production and scavenging, resulting in oxidative stress and cellular damage, aligns with findings for higher plants under cold stress (Hasanuzzaman et al., 2020). Overall, impaired PSII function under cold stress reduced photosynthesis, increased ROS, and compromised antioxidant defences, leading to oxidative damage. This shows that mechanisms of cold stress induced bleaching are similar to those under hot stress for hermatypic corals.

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Investigating the influence of positively valenced decorative visuals on knowledge gain and risk perception

Jennifer Lee（筑波大学 生物学類）　指導教員：Matthew Wood（筑波大学 生命環境系）

Introduction

Decorative pictures in multimedia material are visuals aimed at increasing aesthetic appeal rather than providing additional information and context,[1] and have been shown to influence cognitive processes and emotional affect.[1,2,3] Affect refers to the experience of an emotional state separate from mood, and is a key component of attitude[4]. Affect can be measured along the dimensions of activation and valence.[1] Valence is the force of attraction or aversion to a subject and is often described as a scale from positive to negative,[5] while activation is the degree of intensity of the emotion.

Several studies have found positive affect to be beneficial to human engagement, learning, and behavior. For example, adding a positively valenced decorative picture closely related to the subject material led to decreased irrelevant thinking and improved learning.[1] However, these findings are not consistent, and in another study, adding a decorative visual to a fact list decreased knowledge gain and attitude, and increased perceived risk.[6]

In this study, we ask the question: do positively valenced decorative visuals influence knowledge gain and risk perception?

Material & Methods

We performed a 3 x 1 between-groups comparative study with picture type as the independent variable and knowledge gain, perceived risk, and positive attitude as dependent variables. Picture types consisted of positive visual (PosV), neutral visual (NeutV), and no visual (NoV).

Participants were shown an information sheet concerning *Myotis* bats and their interaction with humans. PosV and NeutV groups each contained one picture of a bat. Pictures were relevant to the content and questions in the survey, but did not provide any additional information. Positive and neutral visuals were determined from a preliminary ranking study of nine photos by 28 independent volunteers.

A total of 128 participants were recruited during the study; 28 for the preliminary photo ranking, 98 for the main study. Two participant's data were removed after data screening.

In the main study, participants were randomly assigned to one of the picture types, presented with the information sheet, and asked questions to evaluate their understanding of the information, their perceived risk of bats and diseases they may carry, and positive feelings and attitudes after looking at the information sheet and picture. NoV group respondents were not asked about a picture. All surveys were conducted online using Google Forms.

Results and Discussion

Internal reliability scores of the risk perception and positive attitude questions were acceptable (α=0.810 and 0.860, respectively), confirming the validity of the scales used in this study.

The positive visual used in the PosV group was rated as significantly more positive than the neutral visual in the NeutV group ($\bar{x}_{PosV}$=5.483(SD=1.056), $\bar{x}_{NeutV}$=4.806(SD=1.167), U=357.000, $p < 0.05$), confirming the influence of the visual on participant affect. However, this did not translate to a similar effect in knowledge, risk perception, and positive attitude scores ($H_{knowledge}$=3.950 (p =0.139), H_{risk}=3.259 (p=0.195), $H_{positivity}$=3.575 (p=0.167)).

These results suggest that the positive affect elicited from a positively valenced visual did not influence knowledge gain and perceived risk towards the subject depicted in the visual. This contrasts with previous studies suggesting positive affect from a visual improves knowledge gain and decreases perceived risk. It should also be noted that although participants demonstrated a difference in positive affect when asked only about the visual, the difference in affect did not extend to their attitude towards bats.

It is important to note, however, that effect sizes were low (around f=0.12 for all tests), and achieved statistical power was approximately 0.19, well below the minimum acceptable 0.8. Therefore, there is an unacceptably high risk that these results constitute a false negative. To reduce the possibility of a Type II error and confirm the lack of significant difference between PosV, NeutV, and NoV groups, a greater sample size of upwards of 750 participants in total is required according to *a priori* estimates.

Additionally, this study could be further improved by the addition of more difficult questions evaluating participants' knowledge gain. In the current study, the majority of participants across all groups were able to correctly answer all knowledge questions, resulting in an inability to conclusively determine a difference between groups.

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