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Special Issue: Singularity Biology and Beyond

Commentary and Perspective (Invited)

Search for singularity cells at the onset of brain disorders using whole-brain imaging

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When a very small number of cells have the potential to cause criticality for the whole system, we refer to this rare cells as singularity cells and the phenomena associated with this criticality as singularity phenomena. Although this possibility does not apply to brain disorders in general, these disorders, such as psychiatric disorders, might be attributed to the abnormality of the singularity phenomena and/or singularity cells, in which environmental and acquired factors augment the abnormalities in the activity of a small number of cells. However, it is unclear how such abnormalities affect a wide brain area and alter mental state. To address these issues, we raised the following questions in the present study and aimed to answer them.

I. What is the molecular basis for the characteristics of the singularity cells involved in brain disorders such as psychiatric disorders?
II. What is the molecular basis for the spread of abnormal activity of the singularity cells across a wide range of brain regions?
III. Is it possible to change the mental and behavioral patterns of individuals by manipulating the spatiotemporal activity of the singularity cells?
IV. What is the molecular mechanisms underlying the generation and development of the singularity cells, and what is the starting point for the onset of brain disorders?

The present study remains unexplored in some of these answers; however, we were able to make some discoveries, which are outlined below.

Whole-brain imaging and subsequent quantitative analysis at the cellular/subcellular level, providing information on cell distribution, neuronal connections, activity patterns, etc, are crucial for understanding brain function and dysfunction. However, there were limitations associated with this approach; it took a long time to image an adult mouse brain with adequate resolution, irrespective of whether it was with or without pre-treatment of tissue clearing techniques. Under these circumstances, in collaboration with the researchers listed in Table 1, we have developed the fast and high-resolution

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tissue whole-cell imaging technique (block-FAce Serial microscopy Tomography, FAST) [1]. Because of the high applicability and scalability of FAST, whole-brain fluorescence imaging can be performed across a variety of sample types, including, e.g., fluorescent-reporter animal models, virus-mediated fluorescence labeling, nuclear-staining dyes, retrograde fluorescent tracers and fluorescent lipophilic dyes [2]. Furthermore, based on the activity and structural maps of individual animal samples obtained by FAST, a comprehensive method was developed to compare them in detail between individuals or groups, which renders unbiased quantitative group comparisons of normal and disease-model brain cells [1,2]. Combining FAST with various brain science research methods enables to propose new hypotheses and to find starting points for research. FAST is not only the prototype for some of the functions that comprise AMATERAS but has continued to be improved and upgraded since the Singularity Biology was started as an elemental technology to be implemented in AMATERAS [3,4].

In collaboration with Dr. Shun Yamaguchi at Gifu University, we conducted brain activation mapping using FAST in Arc-dVenus mice [5] subjected to stress and observed that the activated neurons were increased in several brain regions [1]. Machine-learning-based analysis of these brain regions showed that the activation of the claustrum (CLA) is the most significant marker of exposure to acute stressors. However, as the involvement of the CLA in anxiety response caused by mental stress has not been directly examined, we analyzed the issue in detail [6]. Activity-dependent genetic labeling using TRAP2 mice (also called $FOS^{2A\text{-}iCreERT2/+}$ mice) and subsequent manipulation of neuronal functions revealed the role of activated neurons in the CLA under stress conditions. Intriguingly, in the analysis in Arc-dVenus mice, the number of activated CLA neurons above the control after stresses amounted to only less than 1% of the total CLA neurons (less than 0.001% of the entire brain cells) [1]. Although there was a difference in the immediate early genes used, Arc and c-Fos, it was interesting to see what would happen with manipulating such a very small number of neurons. As a result, we observed that activation of the stress-responsive CLA neuronal ensemble three weeks after stress-induced genetic labeling reproduced anxiety-related behaviors. Conversely, silencing of the CLA neuronal ensemble attenuated anxiety-related behaviors and increased resistance to the emergence of chronic stress-induced depression-like behaviors. These results demonstrate that the CLA neuronal ensemble bidirectionally controls stress-induced emotional responses and that inactivation of the ensemble can ameliorate stress susceptibility [6].

We have also worked on other projects to investigate the pathomechanisms of human diseases in the Singularity Biology. Currently, the relationship between singularity cells and *de novo* mutations is entirely unclear, but we have initiated exploratory research into the potential association between singularity cells and *de novo* mutations, specifically focusing on *de novo* mutated genes in patients with neurodevelopmental disorders, including autism spectrum disorder (ASD). We previously participated in the trio-based whole-exome sequencing in sporadic cases in collaboration with psychiatrists and geneticists, and identified 37 genes with *de novo* single-nucleotide variations [7]. One of these genes, *POGZ* has been identified as one of the most recurrently *de novo* mutated genes in patients with ASD, intellectual disability and White-Sutton syndrome; however, as the neurobiological basis for the involvement of *de novo* mutations on POGZ in these disorders remains unknown [8], we analyzed the molecular and cellular phenotypes of these mutations in detail in the Singularity Biology [9]. We showed that the POGZ missense mutation Q1042R impaired the nuclear localization of POGZ and embryonic cerebral cortical development, and a mouse model carrying corresponding *de novo* POGZ mutation recapitulated the pathogenic abnormalities in patients with ASD. FAST-based whole-brain imaging and subsequent electrophysiological study revealed the increased activation of excitatory neurons in the anterior cingulate cortex in the mutant mice after social interaction. In consistent with these findings, we showed that the social deficits in the mutant mice were rescued by administration of a selective AMPA receptor antagonist, NBQX, or an anti-epileptic negative allosteric modulator, perampanel, which is used in clinical practice. In addition, neuronal differentiation was impaired in neural stem cells differentiated from iPS cell lines established from the ASD patient with the POGZ Q1042R mutation. In the current study, we propose that, even if there are developmental abnormalities, ASD can be treated in adult stages [9,10]. Accordingly, the mouse model and patient-derived iPS cells are robust research tools and warrant further research on neurodevelopmental disorders.

Genome-wide studies have shown that copy number variations (CNVs) are associated with a high risk for psychiatric disorders. Among them, particularly, the 3q29 microdeletion probably confers the greatest risk of schizophrenia with odds ratios as high as 40 [11]. The 3q29 CNV, occurs mostly *de novo*, contains approximately 22 protein-coding genes and is also highly associated with ASD, bipolar disorder, and intellectual disability [12]. We showed that 3q29 deletion mice harboring a deletion of the chromosomal region (chr16:31336396-32632800; GRCm38/mm10) corresponding to the human 3q29 region have reduced brain volume, impaired social interaction, and prepulse inhibition deficits, the latter of which was alleviated by antipsychotics [13]. FAST imaging revealed the increased activity of excitatory neurons in the auditory cortex in 3q29 deletion mice after the social interaction. We also observed reduced expression of parvalbumin, a marker of GABAergic interneurons, in the cortex of the mutant mice, suggesting impaired excitation and inhibition balance in the cortex in these mice [13]. Although the molecular and cellular pathogenesis of the abnormalities in 3q29 deletion mice remains unclear, we showed the possible involvement of oxytocin signaling in impaired social behavior in these mice [14].

In collaboration with prominent domestic and international brain researchers, we have participated in several important studies using FAST. In collaboration with Dr. Baljit S. Khakh at UCLA and his colleagues, we addressed all astrocytes in the brain and revealed their whole picture [15]. As a result, we revealed the molecular basis of astrocyte diversity and morphological complexity and unexpectedly discovered several Alzheimer's disease risk genes in the genes related to astrocyte morphology [15]. In collaboration with Dr. Ayako M. Watabe at the Jikei University School of Medicine and her colleagues, we addressed fear-induced suppression of feeding and showed that the parasubthalamic nucleus innervated by the lateral parabrachial nucleus is critically implicated in avoidance behaviors, aversive learning, and suppressed feeding [16]. We have been involved in other studies promoting our understanding of brain mechanisms.

As described above, we have conducted studies that observe all tissue cells unbiasedly without depending on specific hypotheses or established theories. Combining the study with various brain science research methods led to the proposal of new hypotheses and the discovery of starting points for research. Finally, we hope that FAST and AMATERAS will become "technological Singularity," triggering technological changes that contribute to a deeper understanding of biology and the related fields.

Table 1 A03-3 group composition and collaborators in the Singularity Biology

A03-03 group	
Principal Investigator(PI)	Hitoshi Hashimoto (Osaka University)
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A01-2 PI	Takeharu Nagai (Osaka University)
A02-1 PI	Shuichi Onami (Riken)
A03-2 PI	Kazuki Horikawa (Tokushima University)
A03-2 CI	Tatsuya Takemoto (Tokushima University)
A03 PI	Keiko Muguruma (Kansai Medical University)
A03 PI	Yuki Sato (Kyushu University)
A03 PI	Etsuro Ohta (Kitasato University)
Research management team CR	Taro Ichimura (Osaka University)
Research management team CR	Katsumasa Fujita (Osaka University)

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