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論　文　の　内　容　の　要　旨

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論文題目　Study on the regulation of networked activity in neuronal culture

（初代培養細胞を用いた神経回路網の活動調節機構の研究）

Neurotransmitter, such as the major excitatory neurotransmitter L-glutamate, in the developing mammalian central nervous system (CNS), is a double-edged sword: which is indispensable to mediate signal transmission and leads normal neuronal development, in contrast, excessive synaptic glutamate interferes terminating signal transmission and leads neuronal degeneration. Thus, neuronal networks should keep glutamate concentration within a relatively narrow range to hold a balance of these good or evil effects to maintain normal development. However, it can be furthermore hypothesized that glutamate itself might have multiple functions as 1) feedback its own effect, or 2) modulated by other neurotransmitters, or 3) change its own function according to the neuronal network maturation, to minimize glutamate consumption and obtain maximal information effectively.

In this thesis, I performed three experiments, using synchronized and periodical calcium (Ca^{2+}) spikes, which occur without external stimuli in

primary cultured CNS cells. First, I consider each Ca^{2+} spike represents the index of neuronal activity, as Ca^{2+} spikes were resulted from periodic burst firing of action potentials (see chapter 1). I also confirmed that ionotropic glutamate receptors (iGluRs) were involved in this spontaneous Ca^{2+} spikes, thus I deeply pay attention to glutamatergic transmission as essence to maintain Ca^{2+} spikes. Secondly, I consider Ca^{2+} spikes as *in vitro* model of neuronal development, because the frequency of Ca^{2+} spikes changes concomitant with synapses increasing (see chapter 3).

Recently it was revealed that glutamate, without external stimuli, induce the large spatial synchronized and periodical Ca^{2+} spikes in neuronal network in slices of newborn rats, and this activity is maintained until the developmental transition of the GABAergic transmission from depolarization to hyperpolarization(Garaschuk *et al.* 2000). Additionally, it is known that endogenous glutamate induce synchronized and periodical Ca^{2+} spikes in primary cultured neurons obtained from embryonic or neonatal rat cerebral cortex. The phenomenon are occurred also without external stimuli, during the period of *in vitro* neuronal network maturation, resulting from periodic burst firing of action potentials(Leinekugel *et al.* 1997; Wang and Gruenstein 1997; Bacci *et al.* 1999). These Ca^{2+} activities induced by glutamate in developing CNS or neuronal network, can be hypothesized encoding information and playing an important role in neuronal development, such as neuronal differentiation, motility of axonal and dendritic growth cones, and connection patterning(Shatz 1990; Spitzer 1994; Gu and Spitzer 1995; Katz and Shatz 1996; Komuro and Rakic 1996; Feller 1999; Zhang and Poo 2001). For example, the speed of growth cone migration in the developing spinal cord is reported to be controlled by the frequency of spontaneously Ca^{2+} transients: growth cones experiencing a high frequency of Ca^{2+} transients (10–12 times per h) migrate slowly or retract, whereas growth cones experiencing a low frequency of Ca^{2+} transients (0–0.4 times per h) migrate rapidly(Gomez and Spitzer 1999).

I therefore used periodical Ca^{2+} spikes in primary cultured neurons as *in vitro* model of neuronal development, which manage to control glutamate, the dangerous element, and then performed following three experiments focusing on its frequency or the pattern of abolishment.

Chapter 1: The regulation of the frequency of Ca^{2+} spikes in relatively

homogeneous cerebral cortical neurons
Chapter 2: The regulation of Ca^{2+} spikes between heterogenous midbrain neurons: glutamatergic and dopaminergic neurons
Chapter 3: The regulation of Ca^{2+} spikes between heterogenous cells: neurons and astrocytes

In chapter 1, I demonstrate that metabotropic glutamate receptors (mGluRs) were involved in the synchronized Ca^{2+} spikes, in addition to the involvement of iGluRs. I revealed that glutamate-induced signals through mGluRs decreased the frequency of Ca^{2+} spikes, mainly due to the signal through group II mGluR, inactivation of adenylate cyclase-cAMP-PKA signaling pathway. That is, glutamate generates the synchronized Ca^{2+} spikes through iGluRs and modulates simultaneously their frequency through group II mGluR-adenylate cyclase-cAMP-PKA signaling pathway in the present cerebral cortical neuronal network.

In chapter 2, I demonstrate that, in addition to glutamate, endogenous dopamine were involved in synchronized Ca^{2+} spikes in midbrain neuronal network. The regulation of dopamine was distinctly different through two dopamine receptor families, dopamine receptor 1 (D1R) and 2 (D2R): the action of dopamine through D1R or D2R was facilitative or suppressive, respectively, to the Ca^{2+} influx of synchronized Ca^{2+} spikes. I further confirmed that the suppressive effects of D2R were mediated by the regulation of Ca^{2+} influx through L-type voltage-gated Ca^{2+} channel, not through NMDA receptor.

In chapter 3, I demonstrate the importance of astrocytes, predominant glial cell in the CNS, to maintain normal spontaneous Ca^{2+} spikes in primary cultured cortical cells. I present that astrocytes suppressively regulate neuronal Ca^{2+} spikes via glutamate transporter 1 (GLT-1) throughout the neuronal development. However, this regulation of GLT-1 may switch from suppression on Ca^{2+} spike occurrence to suppression on frequency of Ca^{2+} spikes during the formation of neuronal network.

Neurons in during the formation of network appear to add several

functions to one neurotransmitter, for strict regulation of normal neuronal development. Thus glutamate indeed had multiple functions as 1) feedback its own effect, or 2) modulated by other neurotransmitters, or 3) change its own function according to the neuronal network maturation, to minimize glutamate consumption and obtain maximal information effectively. The concept that one neurotransmitter is actually multiple players might further apply to other non-neuronal cell communications especially in the period of development.