Neuroscience of water molecules: a salute to professor Linus Carl Pauling

Tsutomu Nakada

Received: 15 May 2009 / Accepted: 20 July 2009 / Published online: 9 August 2009
© Springer Science+Business Media B.V. 2009

Abstract More than 35 years ago double Nobel laureate Linus Carl Pauling published a powerful model of the molecular mechanism of general anesthesia, generally referred to as the hydrate-microcrystal (aqueous-phase) theory. This hypothesis, based on the molecular behavior of water molecules, did not receive serious attention during Pauling's life time, when scientific tools for examining complex systems such as the brain were still in their infancy. The situation has since drastically changed, and, now, in the twenty first century, many scientific tools are available for examining different types of complex systems. The discovery of aquaporin-4, a subtype of water channel abundantly expressed in glial systems, further highlighted the concept that the dynamics of water molecules in the cerebral cortex play an important role in important physiological brain functions including consciousness and information processing.

Keywords Aqueous-phase theory · Neural net · Kohonen's map · Aquaporin-4 · Brain chip

Introduction

In the early 1990s, the Japanese Ministry of Education, Science, Sports, and Culture (Monbu Sho)¹ launched a revolutionary plan for funding academic science in Japan. The project plan focused support to super select research teams deemed capable of developing into world class centers of excellence (COE), in fields ranging from cosmology to economics. The first five groups selected were announced in 1995. In the following year, in search of the scientific basis of the human mind, the committee decided to establish an inter-disciplinary research institute for complex system brain science. I was recruited from University of California, Davis, to direct this project and established the Center for Integrated Human Brain Science (<http://coe.bri.niigata-u.ac.jp>). The primary efforts of the Center are focused around developing a facility capable of integrating relevant modern technologies useful in the analysis of human brain function, from molecular biology to ultra-high field magnetic resonance imaging (MRI). The principal direction of the center's research activities is an interdisciplinary approach towards a single goal: elucidation of the role of water molecules in brain functionality. The goal coincides with my main career objective, nurtured since my medical student years at University of Tokyo when I discovered Linus Pauling's 1961 ground

---

T. Nakada (✉)
Center for Integrated Human Brain Science, Brain
Research Institute, University of Niigata, 1 Asahimachi,
Niigata 951-8585, Japan
e-mail: tnakada@bri.niigata-u.ac.jp
URL: <http://coe.bri.niigata-u.ac.jp>

---

¹ Currently, Ministry of Education, Culture, Sports, Science, and Technology (Monbu Kagaku Sho).

breaking publication (Pauling 1961). In the 7 years of research, the project has progressed to the stage of testing specific biological hypotheses related to Pauling's legacy, and continues to be supported by the Japanese Government (Monbu Kagaku Sho) as a top priority project under the title "Neuroscience of Water Molecules." This scientific essay introduces an ambitious theory, 'The Vortex Theory of the Brain' (Nakada 2000), the basis of the currently funded project. Along with the experimental proof for certain components of the theory, several testable hypotheses for future experimental designs will be introduced.

Origin

Double Nobel laureate (Chemistry and Peace) Linus Carl Pauling, was deeply intrigued by the fact that the inert gas Xenon was an excellent general anesthetic. His research led him to conclude that the only property common to all anesthetic agents, including Xenon, should be their effect on water crystallization (Pauling 1961).² Unfortunately, his hydrate-microcrystal (aqueous-phase) theory did not receive significant attention. Nevertheless, there is no doubt that Pauling's hypothesis represented a serious paradigm shift in medicine as well as neuroscience which deserves serious examination.

Pauling attempted to apply his theory directly to the dynamics of intracellular water molecules and neuronal membrane potentials. The effort did not yield corroborative results. Retrospectively, this appeared the main reason why the hydrate-microcrystal theory did not receive the nod of approval from the scientific society at large. Furthermore, scientific methodologies during his era were limited and still primarily based on linear concepts. Complex system analysis was still in its infancy. Under that environment, Pauling's hydrate-microcrystal theory was not particularly attractive to the general scientific community (Marinacci 1995).

Needless to say, Pauling's theory of the molecular mechanisms of general anesthesia has tremendous significance on brain science. Should one accept that alteration in the dynamical condition of water molecules to be the basis of general anesthesia, it can be easily deduced that the behavior of water molecules

in the brain should play an important, if not sole, role in creating and maintaining arousal (subjective consciousness). The problem was to find suitable methodologies for approaching this unprecedented powerful concept.

The brain deals with information. Mathematically, information is defined as entropy, as detailed by Claude Shannon in his information theory. One would intuitively consider that the proper method for analyzing functionality of the brain should also be stochastic in nature, i.e., the brain is a complex system. Stochastic methodologies in complex system science, therefore, are the most appropriate methodologies for linking Linus Pauling's legacy to modern brain science.

Brain and self-organization: complex system brain science

Everything which appears spontaneously in our universe comes to be so through self-organization, a representative characteristic of complex system behavior (von der Malsburg 1995). Therefore, it is possible to analyze the brain using the principles of self-organization, and by considering two different self-organization processes, namely, structural and functional.

Cortical self-organization: ontogeny

Ontogenetic development of the cerebral cortex is well described (Rakic 1995). The cells which eventually form the six-layer cortical mantle migrate from the surface of the lateral ventricles, the germinal matrix, following guiding tracts provided by radial glial fibers (Fig. 1). Each migrating cell reaches the surface of the cortex after first passing through all the previously migrated and settled cells, a process analogous to a six-story building constructed beginning from the first story and proceeding upward to the sixth. Following completion of formation of the layers of the cortex, the radial glial fibers disappear. It is easily understood that the determining factor of the gross shape of the brain is the growth pattern of the radial glial fibers. Each radial glial fiber corresponds to a cortical column.

In nature, most of self-organizing structural formation follows the Markovian rule. In order to demonstrate that this principle applies to brain development, we have performed simulation studies for gross

² See simulation at http://coe.bri.niigata-u.ac.jp/content/VTheory_en

Fig. 1 Neuroblast delivery system. *Left* schematic presentation of the process of neuroblast migration. *RGF* radial glial fiber *GM* germinal matrix, *CP* cortical plate. *Right* Scanning electron-microscope picture depicting actual neuroblast migration (Courtesy of Y. Ikuta, professor Emeritus, University of Niigata)

structural organization of the brain (Nakada 2003). The results strongly indicate that the self-organizing process of the brain likely follows the rule of heat convection (Figs. 2, 3). If one accepts that the gross brain shape is indeed defined by a plume type heat convection pattern, then it follows that its biological realization requires only two conditions, namely, (1) heat should constantly escape into amniotic fluid from the core through the surface of the fetal brain; and (2) radial glial fibers have to advance their fiber tips in a direction identical to that of convective heat flow. Whereas the first condition is accepted fact (Schröder and Power 1997; Laburn et al. 2002), the testable hypothesis for second condition can be easily constructed: *Hypothesis I: Radial glial fibers emit small amounts of a gaseous substrate, such as nitric oxide (NO), and advance their fiber tips towards the highest concentration of the emitted gaseous substrate* (Fig. 4). Each fiber tip advances to its destination by following the rule of heat convection governing the particular space in which the tip is located.

Functional self-organization: self-organizing neural net

Research on synaptic plasticity in the cerebellum dramatically advanced the concept of brain function (Ito 2001). The discovery of learning neurons confirmed the existence of the biological counterpart of the

McCulloch and Pitts neuron, an artificial neuron in a neural net. The discovery in turn provided virtual proof for the concept that, similar to artificial neural nets, diverse functionality of the brain can be constructed based on a single functional unit (Fig. 5). The concept of cerebellar learning was further refined by the identification of a biological functional unit often referred to as the *cerebellar chip*.

A simplified representation of the cerebellar chip is shown in Fig. 6. The chip is organized around a single output neuron, the Purkinje cell. Information reaching the cerebellum is first processed by many, so called, pre-processing neurons such as the granular cells. The output of these pre-processing neurons reaches the Purkinje cells via the parallel fibers and form synaptic connections with dendrites of the Purkinje cells. Transmission efficacy of the synapses between parallel fibers and the Purkinje cells is modifiable, forming the basis of synaptic plasticity, and provides the biologic substrate of the cerebellar learning processes. The role of transmission efficacy in the learning process is analogous to the variable weights in the learning process of the McCulloch and Pitts neuron (Fig. 5).

The human cerebral cortex contains more than 100 billion neurons and 10^{14} synapses. Even without regard to the size of the genome, it can be easily concluded that a deterministic blueprint for connectivity of such an enormous number of networks is unrealistic. Therefore, as in the case of structure formation, self-organizing processes must play a significant role in developing functional connectivity of neurons. Nevertheless, unlike structure formation, simulation studies cannot provide virtual proof for functional self-organization. Therefore, the means of determining the self-organizing processes of functional connectivity should rely on probability analysis. Among the many neural net algorithms, the concept generally referred to as Kohonen's self-organizing map represents the most probable candidate algorithm that applies to self organizing functional connectivity in the cerebral cortex (Kohonen 2001).

Kohonen's map is a *non-linear*, two dimensional, self-organizing neural net (Fig. 7). Kohonen's map automatically creates "areas" within the map which can be specifically activated by a corresponding stimulus by applying identical or similar stimuli repeatedly. The algorithm is capable of automatically creating all aspects of functional organization thus far defined in neurophysiology, including the orientation

Fig. 2 Plume type heat convection simulation. Seventy consecutive frames of representative slices of the simulation. *Left upper* to *right lower* frames demonstrate clear development of structural complexity over time. Note after a certain point in time, approximately frame 60 in this simulation, no further significant structural change is appreciated

Fig. 3 Heat convection and brain shape. A representative example of simulation results (frame 66 of Fig. 2). Due to imperfection of the selected initial conditions, the simulated image (simulation) is not wholly identical to actual brain

(Schematic). Nevertheless, the striking similarities in structural detail are demonstrated, strongly indicating that brain organization indeed follows the self-organization schema of free convection

of the columns of the visual cortex, utilizing only repeated stimuli. It follows that functionally, Kohonen's map meets not only a necessary, but also sufficient condition to be an algorithm of the cerebral cortex (Arbib 2002).

From the view of system organization, Kohonen's net is actually a two dimensional version of the McCulloch and Pitts neuron. While the McCulloch and Pitts neuron learn individually, neurons in the Kohonen's map learn simultaneously as a group. Given

that the biological realization of the McCulloch and Pitts neuron is generally accepted to be the *Cerebellar Chip*, the biological realization of the Kohonen's map would be in the form of the proposed "*Brain Chip*". Accordingly, the second testable hypothesis is formulated: *Hypothesis II : Each cortical column represents a functional unit referred to as Brain Chip* (Fig. 8). This hypothesis actually leads to another testable hypothesis regarding the role of glial organization as will be discussed below.

Fig. 4 *Hypothesis I*. Schematic presentation of growth pattern of a radial glial fiber, the first testable hypothesis. In this schema, the gaseous substrate is defined as nitric oxide (NO). The tip of the growing radial glial fiber emits a gaseous substrate (NO in this figure), which flows based on the heat convection schema. The radial glial fiber grows in the direction of NO flow. *GM* germinal matrix, *RGF* radial glial fiber, *RGC* radial glial cell

Fig. 5 McCulloch–Pitts classical linear model of the neuron. At given time t , input signals $x_i(t)$ reach the synapses. Each input is transmitted through the synapse to the neuron after each has been modified by weight w_i . When the sum of the inputs reaches threshold θ , the output of the neuron at time $t + 1$, $y(t + 1)$, will become 1. $y(t + 1) = \begin{cases} 1 & \text{if } \sum_i w_i x_i(t) \geq \theta \\ 0 & \text{if } \sum_i w_i x_i(t) < \theta \end{cases}$

Aquaporin-4 and water homeostasis in the brain

In the process of searching for proof for *Hypothesis II* above, one would immediately realize that biological realization of the *Brain Chip* cannot be achieved based on neuronal connectivity only. There are no known neural connections which can carry learning

Fig. 6 Cerebellar chip. Functionally, the cerebellum is now considered to be an organ formed collectively by identical functional units. The individual unit is referred to as “cerebellar chip”, in analogy to a computer chip. Each cerebellar chip has a single output neuron, the Purkinje cell. Information to the cerebellum is first processed by numerous preprocessing neurons, such as the granular cells, and eventually reaches the Purkinje cells via parallel fiber (PF) input. The transmission efficacy of the synapses between parallel fibers and Purkinje cells is modifiable, forming the basis of synaptic plasticity, and provides the substrate of the cerebellar learning process. The outcome of cerebellar system output is examined in other systems, and error signals are fed back to each Purkinje cell if the outcome is undesirable. These error signals are carried by the climbing fiber (CF) and provide the learning trigger signal (LTS) for the corresponding Purkinje cells. Once a desirable outcome is achieved, the error signals will cease, and the learning process will be put on hold similar to the situation where the hold command is given to the McCulloch and Pitts neuron

Fig. 7 Kohonen's Self-organizing Map. One of the most successful neural net applications for the creation of associative memories similar to that observed in brain, in vivo, is the self-organizing map (SOM) initially introduced by Kohonen, a non-linear method based on unsupervised learning processes. Any point on the two dimensionally spread neural lattice can be excited. For each input $\mathbf{x}$, the learning processes are confined to a local group of neurons centered at site s , where maximum adaptation occurs. The adaptive changes will decay according to their distance from the center (Neighborhood kernel) in Gaussian fashion. $h_{rs} = \exp\left(-\frac{\|\mathbf{r}-\mathbf{s}\|^2}{\sigma^2}\right)$. Assuming active, non-linear forgetting, ε , occurs, the learning rule of each synapse can be given as: $w_r^{(\text{new})} = (1 - \varepsilon \cdot h_{rs})w_r^{(\text{old})} + \varepsilon \cdot h_{rs} \cdot \mathbf{x}$

Fig. 8 *Hypothesis II*. Schematic representation of Brain Chip concept, the second testable hypothesis. The brain chip is a two-dimensional cerebellar chip where multiple pyramidal cells receive learning trigger signals (blue arrows) simultaneously. In this schema two sets of six pyramidal cells are considered. Neurons of each group will receive LTS simultaneously. The signal intensity of LTS will diminish according to

the distance from the center and therefore, neurons in the near group (red dotted line) will receive LTS more frequently than those in the distant group (purple dotted line). This provides a “weighted learning” process necessary for creating a unit which conforms to the algorithm equivalent to Kohonen’s map. LTS learning trigger signal, NNG near neuron group, DNG distant neuron group

trigger signals (LTS) simultaneously to each group of neurons in a *Brain Chip* configuration. Should each column conform to a single *Brain Chip* unit, there must exist a system that is related to the structural self-organization of these columns. The Vortex Theory of the Brain and Brain Chip hypothesis call for radial glial fibers and resultant glial matrix to fill this role (Nakada 2000, 2004).

A complete discussion of the Vortex theory is above the scope of this brief review. However, the essence of the Vortex Theory can be highlighted by another testable hypothesis: *Hypothesis III: There should be a “dry area” within the cortex created by the glial matrix.* The key structure which strongly supports this hypothesis was discovered in the late twentieth century, namely, aquaporin-4, a water channel widely expressed in glial cells of mammalian brains (Rash et al. 1998).

The organizational characteristic of the glial system is its matrix formation, which is an effective means of establishing compartments of different contents, and hints to the fact that the brain likely maintains two different types of extracellular space. The conventional extracellular space is filled with extracellular fluid, the volume of which is known to be

significantly small in brain compared to other organs. Indeed, conventional extracellular space filled with extracellular fluid is found only in certain specific structures, e.g., the surroundings of synapses and the nodes of Ranvier, where astrocytes tightly seal off these areas, as if to prevent any leakage of extracellular fluid (Fig. 9). Intracellular space and conventional extracellular space is filled with fluid of different ionic environments. Therefore, one of the most attractive ideas for the properties of the second extracellular space is differential water contents, namely, dry space, i.e., gas containing space, providing the last testable hypothesis: *Hypothesis IV: Aquaporin-4 creates and maintains dry matrix spaces.*

If one accepts *Hypothesis IV* as true, two long-standing mysteries regarding the brain can immediately be solved, namely, (1) why brain has a low specific gravity; and (2) why brain has only a limited volume of conventional extracellular space. Furthermore, it is easy to visualize that a matrix structure containing dry spaces is the basic configuration of Styrofoam, a widely used protective packaging material. Electron micrographs of the glial matrix system and Styrofoam indeed show striking similarity (Fig. 10). Direct proof waits for further refinement of

Fig. 9 *Hypotheses III and IV.* Left schematic presentation of an astrocyte and its principal processes. Astrocytes possess all the structural significance necessary for the anatomical realization of the hypothesis. The key elements include: (1) electron rich layer formed by the principal processes just beneath the pia mater; (2) two compartments of extracellular space segregated by principal processes; and (3) assembly.

“D” indicates “dry area”, the second extracellular space of *Hypothesis III*. See text for details. *a* astrocyte, *b* neuron, *c* axon bundle, *d* vessel, *e* neighboring astrocyte, *f* electron rich layer, *g* assembly. Right Electron-microscopic picture depicting aquaporin-4 rich astrocyte processes. Upper glia limitans, Lower perivascular area. (Courtesy of J. Neeley. Johns Hopkins University)

in vivo microscopic techniques capable of testing uneven water proton density distribution within the brain (Nakada et al. 2008a, b).

In spite of the considerable number of ongoing investigations on the physiological and pathophysiological properties of aquaporin-4 in the brain, direct evidence that AQP-4 plays a direct role in information processing within the cerebral cortex remains elusive. Nevertheless, evidence is steadily accumulating that aquaporin-4 and, hence of water molecular

dynamics in the brain is an indispensable factor for physiological neural-flow coupling associated with brain activation (Huber et al. 2007, 2009; Huber and Nakada 2008; Kitaura et al. 2009). The recent discovery that both antiepileptic drugs (AEDs) and leading anti-migraine drugs, triptans, are effective aquaporin-4 inhibitors (Huber et al. 2007, 2009) provide further tangible clues in support of the concept that cortical processing is closely related with brain water homeostasis.

Fig. 10 Glial matrix as a biological Styrofoam. Electron-microscopic picture of Styrofoam (a) and glial matrix (b) (Courtesy of Y. Ikuta, professor Emeritus, University of Niigata)

Conclusion

Linus Pauling left a substantial legacy for brain science by putting forth/proposing that the molecular mechanism of general anesthesia is strongly related with dynamical behavior of water molecules in the brain. Modern brain science has steadily accumulated evidence that not only neurons, but also glial cells play an essential role in creating and maintaining brain cortical functions. The discovery of aquaporin-4, which is abundantly present in brain glial cells, further accentuated the as yet faint concept of the functional role of water molecules in the brain. Nevertheless, one can be assured that brain science is about to enter the era of water as certainly as, astronomically, earth moved from the Pisces era to Aquarius (Nakada 2006).

Acknowledgments The concept was presented in part at various national and international scientific meetings including Neuroscience 2006, Atlanta, GA, USA, October 14–18, 2006, and 5th World AMN-Congress, Düsseldorf, Germany, May 10–12, 2007. The study was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology (Japan), University of Niigata Suzuken Memorial Foundation, and the Uehara Memorial Foundation.

References

- Arbib MA (2002) The handbook of brain theory and neural networks, 2nd edn. The MIT Press, Massachusetts
- Huber VJ, Nakada T (2008) Aquaporin 4 as a therapeutic target: a first look. *Drugs Future* 33:897–909
- Huber VJ, Tsujita M, Yamazaki M, Sakimura K, Nakada T (2007) Identification of arylsulfonamides as aquaporin 4 inhibitors. *Bioorg Med Chem Lett* 17:1270–1273
- Huber VJ, Tsujita M, Kwee IL, Nakada T (2009) Inhibition of aquaporin 4 by antiepileptic drugs. *Bioorg Medic Chem* 17:418–424
- Ito M (2001) Long-term depression: characterization, signal transduction, and functional roles. *Physiol Rev* 81:1143–1195
- Kitauro H, Tsujita M, Huber VJ, Kakita A, Shibuki K, Sakimura K, Kwee I, Nakada T (2009) Activity dependent glial swelling is impaired in aquaporin-4 knockout mice. *Neurosci Res* 64:208–2121
- Kohonen T (2001) Self-organizing maps, 3rd edn. Springer, Heidelberg
- Laburn HP, Faurie A, Goelst K, Mitchell D (2002) Effects on fetal and maternal body temperatures of exposure of pregnant ewes to heat, cold, and exercise. *Appl Physiol* 92: 802–808
- Marinacci B (1995) Linus Pauling in his own words. Touchstone, New York
- Nakada T (2000) Vortex model of the brain: the missing link in brain science? In: Nakada T (ed) Integrated human brain science. Elsevier, Amsterdam, pp 3–22
- Nakada T (2003) Free convection schema defines the shape of the human brain: brain and self-organization. *Med Hypo* 60:159–164
- Nakada T (2004) Brain chip: a hypothesis. *Magn Resonance Med Sci* 3:51–63
- Nakada T (2006) Water molecules in the brain. Kinokuniya, Tokyo
- Nakada T, Matsuzawa H, Kwee IL (2008a) High resolution imaging with high and ultra-high-field MRI systems. *NeuroReport* 19:7–13
- Nakada T, Matsuzawa H, Igarashi H, Fujii Y, Kwee IL (2008b) *In vivo* visualization of senile plaque like pathology in Alzheimer's disease patients by MR microscopy on a 7T system. *J Neuroimag* 18:125–129
- Pauling L (1961) A molecular theory of general anesthesia. *Science* 134:15–21
- Rakic P (1995) Radial glial cells: scaffolding for brain construction. In: Kettenmann H, Ransom BR (eds) *Neuroglia*. Oxford University Press, New York, pp 746–762
- Rash JE, Yamamura T, Hudson CS, Agre P, Nielsen S (1998) Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proc Natl Acad Sci* 95:11981–11986
- Schröder HJ, Power GG (1997) Engine and radiator: fetal and placental interactions for heat dissipation. *Exp Physiol* 82: 403–414
- von der Malsburg C (1995) Self-organization and the brain. In: Arbib MA (ed) The handbook of brain theory and neural networks. MIT Press, Cambridge, pp 840–843