observed in the absence of any activation (data not shown). To confirm the complete replication of the virus leading to the production of progeny viruses, viral DNAs in the culture supernatants of the infected cells were quantified by real-time PCR. The HHV-6 DNA released from both the T cell fractions was greatly increased only when the cells were stimulated with CD3/CD28 beads (Fig. 3B). The difference of HHV-6 DNA quantity between two cell populations would be related to cell number propagated by activation as shown in Fig. 2. These results indicated that HHV-6 could replicate completely in these T-cell subsets.

When FoxP3-positive cells in both the T-cell subsets from freshly isolated PBMCs were detected by an immunofluorescence test, approximately 60% of $CD4^+CD25^{high}$ T cells were Ag positive, while a total of less than 1% $CD4^+CD25^-$ T cells were Ag positive (data not shown). Therefore, we examined whether HHV-6 can replicate in FoxP3-positive and/or FoxP3-negative T cells in the $CD4^+CD25^{high}$ fraction. As seen in Fig. 4, HHV-6 gB was detected in both the types of T cells, indicating that the virus can replicate in both $T_{reg}$ and non-$T_{reg}$ cells.

*Cytokine Production from $CD4^+$ T Cell Fractions*

We examined the effects of HHV-6 infection on cytokine productions from $CD4^+CD25^{high}$ and $CD4^+CD25^-$ fractions. Freshly isolated cells did not produce anycytokines if they were not stimulated (data not shown). However, upon stimulation, both the $CD4^+CD25^{high}$ and $CD4^+CD25^-$ T cells secreted significant amounts of IL-2 and IL-10, and IL-2, IL-10, and IFN-γ, respectively (Fig. 5). In the $CD4^+CD25^{high}$ T cells, IL-2 excretion decreased on day 2 but that of IL-10 on days 1 and 2 was fairly constant (Fig. 5A, B). Similar release kinetics were observed for IL-2 and IL-10, and IFN-γ was constantly produced by $CD4^+CD25^-$ T cells (Fig. 5C). When the $CD4^+CD25^-$ T cells were infected with HHV-6, their IL-2, IL-10, and IFN-γ productions were significantly enhanced (Fig. 5). In contrast, modest and no enhancement of IFN-γ and IL-2, respectively, was observed in the $CD4^+CD25^{high}$ T cells; however, IL-10 secretion was significantly augmented. No basal production of IL-4 was detected under any conditions (data not shown).

## Discussion

This study demonstrated for the first time that HHV-6 could completely replicate in both $CD4^+CD25^{high}$ and $CD4^+CD25^-$ T cells. Furthermore, in the $CD4^+CD25^{high}$ T-cell fraction, both $T_{reg}$ cells (FoxP3 positive) and non-$T_{reg}$ cells (FoxP3 negative) permitted viral replication. We also found that virus infection enhanced the production of both Th1- and Th2-type cytokines from $CD4^+CD25^-$ T cells; however, only Th2-type cytokine release was augmented from viral-infected $CD4^+CD25^{high}$ T cells.

Several reports have demonstrated that $T_{reg}$ cells affect functional immunity to microbes. There are reports of an enhanced $CD8^+$ T-cell response to the herpes simplex virus type 1 (HSV-1)-specific peptide in $T_{reg}$ cell-depleted mice, and that $T_{reg}$ cells suppressed anti-human immunodeficiency virus (HIV) immunity (1, 38). Furthermore, some microbes were reported to modulate $T_{reg}$-cell functions directly or indirectly. Lundgren et al. have shown that *Helicobacter pylori*-infected individuals have impaired memory $CD4^+$ T-cell responses that are linked to the presence of pathogen-specific $T_{reg}$ cells (25). Yamano et al. revealed that human T lymphotropic virus type 1 (HTLV-1) is significantly more infectious to $T_{reg}$ cells than other $CD4^+$ T cells; thus, HTLV-1 causes autoimmune diseases by impairing the functions of $T_{reg}$ cells (43). In contrast, elevated $T_{reg}$ cell function has also been reported following HSV-1 infection (38). Multiple lines of clinical and experimental evidence suggest that HHV-6 may act as an immuosuppressive agent. For example, fatal immune suppression associated with disseminated HHV-6 infection and defective Ag-specific T lymphocyte proliferation during episodes of HHV-6 reactivation or reinfection have been reported (12, 21). Although the mechanisms of these immune suppressions are largely unknown, several types of evidence have been reported. These include the inhibition of IL-12 p70 production by macrophages (36), defective Ag presentation and maturation of dendritic cells (20), and aberrant cytokine production (3, 11, 12, 35). On the other hand, autoimmune diseases such as fulminant type 1 diabetes mellitus and multiple sclerosis have been linked to HHV-6 infection (7, 14). Since there is no relevant method to isolate only live $T_{reg}$ cells and since HHV-6 causes $CD4^+$ T cell lysis after 3–4 days of infection, we examined cytokine productions from these cells within 2 days after infection to estimate the participation of $T_{reg}$ cells in immune modulation. HHV-6 enhanced the secretion of both Th1 and Th2 cytokines (IL-2, IFN-γ, and IL-10) in $CD4^+CD25^-$ T cells; however, only Th2 cytokine (IL-10) production increased slightly without any change in Th1 cytokine excretion in $CD4^+CD25^{high}$ T cells. Arena et al. reported that HHV-6 up- and downregulated IL-10 and IFN-γ, respectively, in PBMCs induced by lipopolysaccharide (3). Moreover, IL-2 release from PBMCs and $CD3^+$ and $CD4^+$ T cells were all inhibited by virus infection. In addition to the cells used, the type of viral strain

might also be the cause of different release patterns of IFN-γ and IL-2. Arena et al. used the variant A HHV-6 strain, while we utilized the variant B strain. Hence, our findings are difficult to compare with these authors' reports due to the abovementioned factors. Nevertheless, our results implied that the $CD4^+CD25^{high}$ T-cell subset, in which $T_{reg}$ cells comprise more than half the cell population, would shift their immunity toward the Th2 type by IL-10 excretion. In contrast, the role of the $CD4^+CD25^-$ T cell population in viral immunomodulation remains to be studied in detail as these cells also produce Th1-type cytokines in addition to IL-10. IL-10 excretion from both cell populations were more abundant than IL-2 and IFN-γ production. In addition to its direct immune suppressive activity, IL-10 is important to induce Tr1 cell (6, 24). Therefore, enhanced excretion of IL-10 by HHV-6 infection may result in induction of Tr1 cells, leading to stronger immune suppression, and this suppression will make the virus propagation easier. Furthermore, IL-2 can activate T cells, and it is essential for HHV-6 replication (15), so that enhanced IL-2 production will also facilitate the virus propagation.

In humans, $CD4^+CD25^+$ T cells secreted IL-10 but not IL-2, IL-4 or IFN-γ (19). However, other reports revealed IL-4 and IFN-γ production in addition to IL-10 production from $CD4^+CD25^+$ T cells, while IL-2 was not excreted from these cells (10, 23). The marked difference between the results of the abovementioned studies and our study is regarding IL-2 production. Presumably, this may be due to the differences in the T-cell isolation methods used. Using FACS, we could collect only $CD4^+$ T cells that expressed high levels of CD25; however, the authors of the abovementioned studies used immunomagnetic beads for isolation, which cannot exclude T cells with low CD25 expression. Although Baecher-Allan et al. investigated cytokine production using $CD4^+CD25^{high}$ T cells sorted by FACS similar to our system, they found that neither IL-10 nor IFN-γ was secreted (4). We also observed very low, if any, secretion of IFN-γ from $CD4^+CD25^{high}$ T cells, but IL-10 was significantly produced unlike in their findings. This may be attributed to the stimulation formulas that may affect the strength of cell stimulation. Although they used plate-bound anti-CD3 either with soluble anti-CD28 or with the addition of a low dose of IL-2 (50 units/ml) for the maximum stimulation of $CD4^+CD25^{high}$ T cells, these stimuli induced modest cell expansion. However, our stimulus using CD3/CD28 beads with a high-dose of IL-2 (500 units/ml) caused extensive cell proliferation, indicating that our stimulus is considerably stronger. Thus, the patterns of cytokine production could vary according to the strength of cell stimulation.

The cell tropism of HHV-6 has been analyzed *in vitro* and *in vivo*, and it has become apparent that $CD4^+$ T cells permit its complete replication; however, no further attempts have been made to study viral tropism of any $CD4^+$ T-cell subsets thus far. Since one of the recent advances in immunology is the discovery of the new $CD4^+$ T cell subset $T_{reg}$, we attempted to investigate whether $T_{reg}$ cells permit viral growth. In fact, there are reports of the preferential infection of $T_{reg}$ cells by HIV (30). Following the infection of $CD4^+CD25^{high}$ T cells by HHV-6, both the number of Ag-positive cells and progeny viruses produced by the infected cells increased. These results suggested that $T_{reg}$ cells might permit complete HHV-6 replication. However, it is likely that the $CD4^+CD25^{high}$ T cell fraction comprises $T_{reg}$ and non-$T_{reg}$ cells, as shown in Fig. 4, that have been found to be either FoxP3 positive or FoxP3 negative. FoxP3-expressing cells comprised approximately 60% of the population. Therefore, the possibility that non-$T_{reg}$ cells in the subset might selectively allow HHV-6 replication, while $T_{reg}$ cells do not, remains to be studied. We attempted to study this topic in greater detail by a single-cell assay. The results revealed that HHV-6 can replicate in most types of $CD4^+$ T cells, including $T_{reg}$ cells. In addition, both the $CD4^+CD25^+$ and $CD4^+CD25^-$ T cell populations of PBMCs isolated from ES patients in the acute phase contained comparable amounts of HHV-6 genomes (data not shown). This suggested that $T_{reg}$ cells have good susceptibility to viral infection *in vivo*. Since HHV-6 utilizes CD46, a ubiquitous membrane cofactor protein on T cells, as a receptor for infection (34), we examined the CD46 expression on each $CD4^+$ T-cell subset. As expected, more than 99% of cells in both populations were CD46 positive (data not shown).

It is known that $CD4^+CD25^{high}$ T cells do not propagate by standard T-cell stimulation, such as that with plate-bound anti-CD3 and soluble anti-CD28 Abs with IL-2. This condition is the so-called "anergic state." However, the anergic state was abrogated in the presence of crosslinked anti-CD3 and anti-CD28 Abs together with a high dose of IL-2; this resulted in efficient cell propagation (16, 17). Although we obtained a large number of $CD4^+CD25^{high}$ T cells by the same treatment, the cells still retained their anergic features because their expansion capacity was lower than that of $CD4^+CD25^-$ T cells (Fig. 2). It has been shown that expanded $T_{reg}$ cells retain their phenotypic and functional features (17).

As reported by Hori and colleagues, the FoxP3 protein is a key transcriptional factor conferring $CD4^+$ T cells with regulatory functions (18). Furthermore, the

forced expression of FoxP3 conferred peripheral $CD4^+CD25^-$ T cells with suppressor functions (13). Thus, it is believed that FoxP3-expressing $CD4^+$ T cells are $T_{reg}$ cells. It has been reported that FoxP3 is expressed in activated $CD4^+CD25^-$, $CD4^+$, and $CD8^+$ T cells (31, 40). However, we did not observe acquisition of the protein in activated $CD4^+CD25^-$ T cells (data not shown). This may due to the difference in assay systems or cells used; the Western blot technique (40) and cloned T cells (31) were used in their studies. Since Western blot analysis measures the total amount of protein in whole cells, it is not clear whether the upregulation of FoxP3 protein expression implies an increase in the number of protein-positive cells or an increase in the protein quantity in a single cell. Furthermore, cloned cells did not, of course, represent a particular cell population. On the other hand, we used single-cell analysis to accurately determine the number of FoxP3-positive cells.

In conclusion, our data indicated that HHV-6 could replicate in both $T_{reg}$ and non-$T_{reg}$ cells in the $CD4^+CD25^{high}$ as well as in $CD4^+CD25^-$ T-cell subsets. Further, virus-infected $CD4^+CD25^{high}$ T cells shift their antiviral immunity toward Th2 dominance by producing IL-10. However, the role of viral infected $CD4^+CD25^-$ T cells remains obscure.

We thank Drs. Kozaburo Hayashi and Hiroko Tsutsui for their helpful opinions and critical reading of the manuscript. This research was supported by a Grant-in-Aid for Researchers, Hyogo College of Medicine.

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MINIREVIEW

Trends in Glycoscience and Glycotechnology
Vol.19 No.106 (March 2007) pp.99–112

# Glycobiological Approach to Understanding Neural Plasticity

## 神経の可塑性における糖鎖の役割

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*Key Words*: *neural plasticity, synaptic plasticity, carbohydrate*

**Abstract**

Neural plasticity is necessary for the expression and maintenance of higher brain functions. While most of the experimental approach to the plasticity still remains phenomenalism, analyses at the molecular level have gradually progressed, especially concerning synaptic plasticity. The analyses revealed that several carbohydrate structures play important roles in the synaptic plasticity. This review describes the roles of 5 of these species of carbohydrates.

**要　約**

神経の可塑的変化は、脳の高次機能の発現と維持に必須の反応であるが、ほとんどの可塑的変化は、いまだ現象論に留まっている。その中において、海馬を中心としたシナプス可塑性については、ずいぶんと分子レベルの解析が進んできており、特定の糖鎖構造が重要な役割を演じていることが明らかとなってきた。そこで本稿では、5種の糖鎖構造に焦点を絞り、シナプス可塑性への関わりについて解説する。

### A. Introduction

The brain comprises a sensory system, a motor system, a limbic system for learning and memory, and a homeostasis system related to body temperature and reproduction. These systems communicate with each other via a complex neural network to express higher brain functions. The neural circuitry is not complete throughout life, with the addition, revision, and maintenance of information over long periods causing the network to change. Such phenomena are known collectively as neural plasticity, comparable to the plasticity of clay. Neural plasticity occurs in various neural regions during (i) development of the neural circuitry in the fetus, (ii) physical and mental development in children, (iii) learning and memory, (iv) breeding, and (v) regeneration following neural degeneration. Several representative examples involving the auditory system are described.

Research has progressed psychophysically concerning the discrimination of frequency, loudness, pitch, tone, and source localization of sound, and it has recently been revealed that the acquirement of source localization, at least is related with neural plasticity. When spectacles were embedded in the optic tectum of juvenile barn owls, the auditory space map in the external nucleus of the inferior colliculus shifted according to the optic displacement of the prisms and induced changes in interaural timing difference (ITD), one of the most important cues for sound localization. After adapting the owls with a prism in the optic tectum, ITD tuning shifted to a long time ($\mu$s). Removing the prism after adaptation led to the tuning returning to normal, short time. Embedding

### A. 序　論

脳は、感覚系、運動系、学習記憶に代表される辺縁系そして、体温や生殖に関係した生体調節系、に分類することができるが、実際には、これらの系（システム）は、複雑に入り組んだ神経回路網によりお互い連絡し、中枢としての脳の高次機能を発現する。この神経回路網は、厳密には完成された形というものはなく、記憶・学習に代表されるように、長期にわたる持続的な情報が加算され保持されることにより、新たな回路が形成・維持され、時に変化し、修正されるものである。こうした神経回路に生じる現象を粘土細工にたとえて、“神経が可塑性を獲得する”という。そして様々な神経の可塑的変化は、(i) 発生過程を含む、脳の回路発達時、(ii) 成長期における心身の発達時期、(iii) 学習記憶時、(iv) 繁殖期、そして (v) 神経損傷に伴う再生時に、様々な神経領域で生じることがわかってきた。では、具体的にどの様な現象が存在するのか、ここでは聴覚系を例に挙げて幾つか記述する。

これまで、音の周波数、音の大きさ・高さ、音色の識別、音源定位（どこから音が聞こえるか）の獲得について、心理物理学的に研究が進められてきたが、近年の神経科学の進歩により、少なくとも音源定位が神経可塑性に関わることがわかってきた。メンフクロウの幼鳥脳の視蓋にプリズムを埋め込み、視覚に変化を起こすと、視覚と聴覚の知覚にずれが生じ、音源定位の手がかりのひとつ、両耳間時間差（ITD）が変化する。プリズムで適応させた幼鳥から、いったんプリズムをはずすと、ITDは短縮するが、再びプリズムを埋め込むと、当初埋め込んでいた時の時間差に戻る。すなわち、メンフクロウは、幼鳥の発達時に得たプリズム適応を保持（記憶）していたこ

the prism again led to a shift of ITD tuning as when the prism was first embedded. This showed that juvenile owls memorize prism adaptation acquired developmentally (1,2). The sensitive period of the prism adaptation ends as the owls approach sexual maturity, at about 200-250 days old, which suggests that acquirement of sound localization is a neural plasticity occurring in the developmental period. For lots of neural plasticity containing developments of auditory, visual, and motor systems as well as sound localization, the periods required for the acquirement are determined and called critical periods. For example, birds communicate through various songs. Song learning involves two components: song memorization and vocal learning. Juvenile birds memorize songs early in life during a critical period and birds are able to sing by vocal learning following the end of the critical period (3). While the start and termination of critical periods seem to be programmed by genetic information, the molecular mechanisms are little understood.

As an example of adult neural plasticity, nonreproductive female midshipman fish present no response to male vocalizations by the inner ear. However, nonreproductive female treated with testosterone or 17β-estradiol exhibit an increase in the degree of temporal encoding of the frequency content of male vocalizations by the inner ear that mimics the reproductive female's auditory phenotype(4). Furthermore, the response of the inner ear is observed seasonally in the summer but not in the winter (5) and is understood as a neural plasticity in breeding season.

Other examples of adult neural plasticity involve neural diseases. For example, it is known that synapses form and neurites develop following brain ischemia and trauma. In addition, neural stem cells in the olfactory ventricle, the lateral ventricle, and the subgranule cell layer of the dentate gyrus develop as new neural cells, which are involved in regeneration. In addition, epilepsy is a disease involving abnormal neural plasticity and repetitive seizures caused by the overloaded discharge of neuronal cells. Finally, there have been reports concerning adult neural plasticity caused by neural diseases.

Neural plasticity is evaluated based on morphological and physiological observations at cell biological level. So far, the synaptic plasticity existing between neurons and between neurons and effector cells has been extensively investigated. It is thought that the physiological activity in neural circuits reacts and changes depending on the condition of synapses. Based on the notion that "two synaptically coupled neurons wire together more strongly, when neurons can fire and fire together (paraphrase of Hebb's postulation 1994)", the molecular cascade and signal transmission in the synaptic region lead to neural plasticity *in vivo* and this has been investigated with a focus on the hippocampus (6,7). For

とになる (1、2)。このプリズム適応は、生後 200-250 日齢に成長したフクロウには起こらない。この日齢は性成熟期に当たることから、音源定位の獲得は子供の時期におこる、神経の可塑的変化であることがわかる。この音源定位に代表されるように、聴覚、視覚、運動発達等に含まれる幾つかの能力は、神経可塑性の獲得時期が決まっており、その時期を臨界期という。例えば鳥は様々なさえずりでコミュニケーションをおこなっているが、臨界期に当たる時期に歌を聴き覚えるが、臨界期を過ぎると、歌を覚えることができない。その一方、さえずりの練習（学習）は臨界期を過ぎていても、学習が成り立ち、うまく歌えるようになる(3)。こうした臨界期のスタートや終了時期の決定は、それぞれ遺伝子情報にプログラムされていると考えられるが、その分子メカニズムは、今のところほとんどわかっておらず、今後の解析に期待される。

次に成熟後に神経可塑性を獲得する例として、不妊雌魚の内耳からの電気信号は、雄の求愛の発声周波数に反応しないが、この不妊雌魚にテストステロンや１７βエストラジオールを与えたところ、内耳は反応し、生殖能力をもつ雌の聴覚表現型を示すようになったという報告がある (4)。またこの内耳の反応は、夏にのみ観察され、冬には消失することから (5)、繁殖期に関連した神経可塑性である事が知られている。

さらに、成熟後に神経可塑性を獲得する別の例として、疾患に関わる可塑的変化も挙げられる。例えば、脳虚血や外傷等により神経損傷を受けた後、新たに軸索をのばし、シナプス結合を形成することが知られている。また、嗅脳室、側脳室、及び海馬歯状回下顆粒細胞層に存在する神経幹細胞が、新たな神経細胞へと分化し、再生に関与することも、可塑性の獲得としてとらえられている。またてんかんは、脳虚血や外傷を含む様々な原因がもとになり、異常な神経の可塑的変化を遂げた結果、神経細胞の過剰な放電に由来する反復性の発作を生じる疾患であり、このように脳の疾患が原因で生じる神経の可塑的変化も多く報告されている。

次に細胞生物学的な見方をすれば、神経可塑性は、形態学的及び生理学的な観察に基づき判定される。そして今日まで、特に、ニューロン間あるいはニューロン－効果器細胞間に存在するシナプスの可塑性に注目した解析が行われてきた（シナプス可塑性)。これまで、神経回路網内での活動は、シナプス結合の活動状況に応じて反応し変化すると考えられている。そして「シナプス結合を示すニューロン間は、発火強度に応じて、シナプス結合をさらに強める（Hebb 則の意訳 1994 年)」という考えを基に、シナプス結合部位での信号伝達や分子カスケードが、神経の可塑的変化をもたらすと考え、記憶の中枢といわれる海馬を中心に研究が進められてきた (6、7)。例え

example, when two stimuli (paired pulses) are applied to the presynaptic nerve, paired-pulse facilitation and paired-pulse depression develop according to differences in the timing of the stimuli (2) (short-term synaptic plasticity). Then, in stimuli, frequency of firing in addition to timing seem to induce long-term synaptic plasticity, that is, in synapses in pyramidal cells of the hippocampus and cerebral cortex, high-frequency continuous spike stimuli at ca. 100 Hz and 1sec induce long-term potentiation (LTP) whereas low frequency ones at ca. 1Hz for 10 min induce long-term depression (LTD). Because the periods of continuous activity increase from seconds to hours, LTP and LTD are models for learning and memory. Furthermore, it has been reported that the size of the spine was increased by LTP and reduced by LTD (8,9). Recently, the study of the molecular mechanisms related to synaptic plasticity has progressed, with reports of the involvement of carbohydrates. This review describes recent findings on synaptic plasticity focusing on the hippocampus, one of the regions of the brain that have been analyzed extensively at the molecular level. First, it describes the "mechanisms of hippocampal LTP" and then the involvement of 5 species of carbohydrates in synaptic plasticity.

## B. Mechanisms of Hippocampal LTP

The hippocampal formation, the center of memory, is linked by unidirectional projections [entorhinal cortex (EC) → dentate gyrus (DG) → CA3 → CA1 → subiculum]. Projections from the entorhinal cortex to the dentate gyrus, from the dentate gyrus to the CA3 subfield, and from the CA3 subfield to the CA1 subfield are known as the perforant-pathway, mossy fibers, and Shaffer collaterals, respectively. Stimuli of the perforant-pathway, mossy fibers, and Shaffer collaterals are recorded in dendrites of granule cells in the dentate gyrus (EC-DG synapse), in the pyramidal cell layer of the CA3 subfield (DG-CA3 synapse), and in the pyramidal cell layer of the CA1 subfield (CA3-CA1 synapse), respectively (10) (Fig. 1). In 1973, Bliss *et al* reported that the response of granule cells in the dentate gyrus to stimulation of the perforant-pathway with high frequency continuous spike stimuli was an induction of LTP for at least 6 hours, in the hippocampus in anesthetized rabbits (11). Then, stimulation of the perforant-pathway was recorded in the dentate gyrus of the hippocampus in un-anesthetized rabbits, resulting in LTP lasting 3 days (12). These results in rabbits indicate that LTP is a model of learning and memory in the hippocampus. Then, the development of a physiological technique with hippocampal slices led to numerous reports of the detection of LTP in DG-CA3 and CA3-CA1 synapses (13), which recently have been more frequent than those with EC-DG synapses. More recently, LTP has been detected in the cerebellum, amygdala, cerebral cortex, and so on.

ば、シナプス前ニューロンに2回刺激を与えた場合(短期シナプス可塑性)、2回の刺激間の時間差に応じて、シナプス後ニューロンの反応が増強する場合(paired-pulse facilitation)と、減衰する場合(paired-pulse depression)がある(2)。この時間差に加えて、発火率の異なる刺激を入力することにより、長期シナプス可塑性が誘導される。海馬や大脳皮質錐体細胞のシナプスなどは、100Hz程度の高頻度連続スパイク刺激を1秒間送ると、長期増強(LTP)が誘導され、1Hz程度の低頻度刺激を10分送ると、長期抑圧(LTD)が起こる。これらの反応持続時間が、短期シナプス可塑性の秒単位から、時間単位に延長することから、記憶学習のモデルとして広く利用されている。さらに、LTPに伴い、後シナプスに位置するスパインが肥大する例や、逆にLTDに伴いスパインが矮小化する例が報告されている(8,9)。近年、こうしたシナプス可塑性に関わる分子メカニズムの解明が進んでおり、糖鎖の関与を示唆する報告も多い。そこで本稿では、シナプス可塑性について、最も分子レベルの解明が進んでいるひとつである海馬を中心に，最近の知見を踏まえて解説する。まずは，海馬LTP発生機序について簡単に解説し、その後構造が特定されている糖鎖のシナプス可塑性への関与について、解説する．

## B. 海馬LTP発生機序

記憶の中枢と呼ばれる海馬は、神経回路の方向が一方向であり、皮質内嗅領(EC)→歯状回(DG)→CA3→CA1→海馬支脚へと刺激が伝わる。そして皮質内嗅領から歯状回へ、歯状回からCA3へ、そしてCA3からCA1へと投射される繊維連絡を、それぞれ貫通繊維路(perforant-pathway), 苔状繊維(mossy fiber), シャファー側枝(Shaffer collateral)という。そして、貫通繊維路，苔状繊維，シャファー側枝に刺激を与えると、それぞれ、歯状回顆粒細胞の樹状突起が位置する分子層(EC-DGシナプス)、CA3錐体細胞層（DG-CA3シナプス)、CA1錐体細胞層(CA3-CA1シナプス)から神経活動の記録を得ることができる(10)(図1)。1973年にBlissらが、麻酔を施したウサギ海馬の貫通繊維路を高頻度刺激し、投射先の歯状回・顆粒細胞樹状突起で記録をとったところ、少なくとも6時間の持続した電位の上昇を観察した。これは、貫通繊維路(軸索)と顆粒細胞樹状突起間でのシナプスにおける長期増強を最初に見たものである(11)。その後無麻酔下のウサギを用いた試みでは、3日間LTPが持続したことから、LTPは海馬における記憶のモデルとしてとらえられるようになった(12)。その後、海馬スライスを利用した電気生理学的な計測の容易さも手伝って、苔状繊維とCA3錐体細胞樹状突起間（DG-CA3）シナプスや、シャファー側枝とCA1錐体細胞樹状突起間(CA3-CA1)シナプスにおいて、LTPが観察されるようになり(13)、今では貫通繊維路より利用される頻度が高くなっている。さらに最近では、小脳、大脳皮質、扁桃体等様々な脳領域でLTPの現象が見つかっており、今では、海馬特有の反応であるというよりは、もう少し一般化したモデルとしてとらえられるようになった。

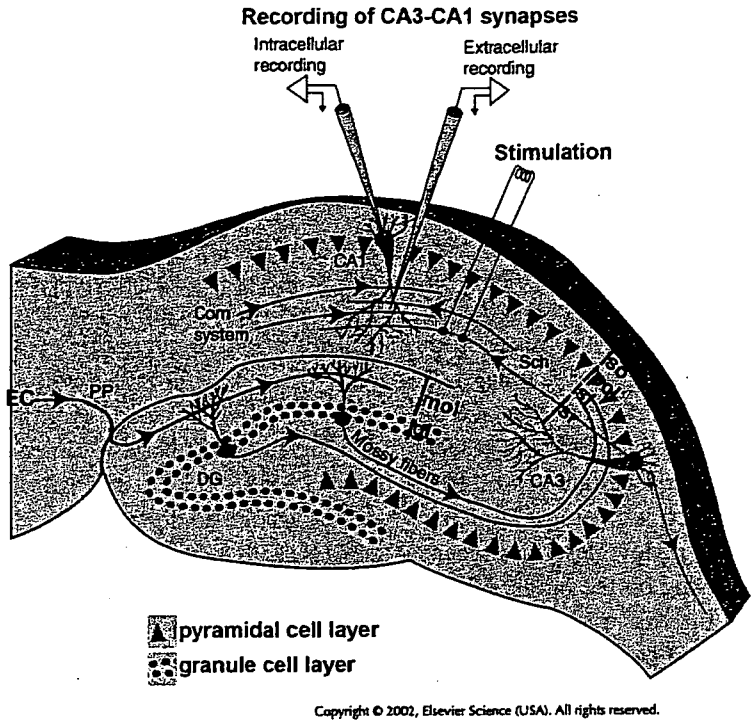




**Fig. 1. A picture of a transverse hippocampal brain slice prepared from the rat and mouse, which was modified slightly from a fig in Fundamental Neuroscience 2nd ed[2]. Com system, commissural system; DG, dentate gyrus; EC, entorhinal cortex; gl, granule cell layer; mol, molecular layer; pcl, pyramidal cell layer; PP, perforant-pathway; Sch, schaffer collateral; sl, stratum lucidum; so stratum oriens; sr, stratum radiatum.**

Next, this review describes the molecular mechanisms of LTP simply. The induction of LTP depends on an increase in the intracellular concentration of calcium ions ($[Ca^{+2}]i$) in some key compartment of pre- and/or postsynaptic cells. The regulation of $[Ca^{+2}]i$ for induction of LTP is controlled by four pathways that have been well studied: *N*-methyl-*D*-aspartate receptor (NMDAR); α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR); calcium influx through voltage-gated calcium channels (VGCCs); and the release of calcium from intracellular stores. The receptors were located in the spine and dendritic shaft (7,14).

While the AMPAR has relatively low $Ca^{+2}$ permeability and AMPAR-mediated conductance is essentially voltage-independent, the NMDAR becomes permeable to $Ca^{+2}$ with the lifting of the $Mg^{2+}$ block but this channel block is relieved by sufficient depolarization of the postsynaptic membrane. Thus, the NMDA-mediated conductance is dependent on voltage. After enough postsynaptic depolarization induces the release of $Mg^{2+}$ from NMDAR, the glutamate released from pre-synapses binds to the NMDAR and causes an influx of $Ca^{+2}$ into dendritic spines on the postsynaptic cell. The $[Ca^{+2}]i$ that increased is thought to activate CaM kinase II ($Ca^{2+}$-dependent

次に、LTP の分子機構について簡単に解説する。LTP の誘導には、前及び後シナプスの細胞内カルシウム濃度 ($[Ca^{+2}]i$) の上昇が必須である。後シナプスへのカルシウムの流入方法は、NMDA 受容体 (NMDAR, N-methyl-D-aspartate receptor); AMPA 受容体 (AMPAR, α -amino-3-hydroxy-5-methyl4-isoxazole receptor); 電位依存的カルシウムチャネル (VGCCs)；細胞内カルシウムストアを介する事が知られており、これら受容体は後シナプスの位置するスパインや樹状突起軸に分布している (7、14)。

AMPAR が $Ca^{+2}$ に対する透過性が低く、電位依存性伝導を示さない受容体であるのに対して、NMDAR は通常 $Mg^{+2}$ により開口がブロックされており $Ca^{+2}$ を透過しない、電位依存性伝導を示す受容体である。グルタミン酸による AMPAR の開口による $Ca^{+2}$ 流入により、後シナプス膜が十分に脱分極すると、NMDAR から $Mg^{+2}$ が離れて、チャネルが開口する。この NMDAR のチャネルの開口と、前シナプスからの $Ca^{+2}$ 放出とが一致したときのみ、$Ca^{+2}$ が後シナプスへ流入する。増加した $[Ca^{+2}]i$ が、スパイン直下の CaM kinase II ($Ca^{2+}$-dependent kinase II) 等のカルシウム依存性のキナーゼを活性化し、その

kinase II), which plays a key role in the induction of LTP. While this progress is known as NMDAR-dependent LTP, LTP not requiring the NMDAR also exists (NMDA-independent LTP) (15-17). For example, in the presence of the competitive antagonist APV (2-amino-5-phosphnovalerate), LTP has been induced in DG-CA3 synapses. The NMDA-independent LTP has been prevented by a blocker of VGCC, showing that VGCC is involved in the induction of LTP instead of NMDAR (15,16,18). Also, it is known that NMDA-independent LTP occurs via VGCC in some of the CA3-CA1 synapses.

Another mechanism for the induction of LTP involves the metabotropic glutamate receptor (mGluR) (19). Induction of LTP was prevented by a blocker of mGluR, MCPG [(+)-α-methyl-4-carboxy-phenylglycine], in the CA3-CA1 synapse, while LTP was induced by addition of MCPG following highly frequent stimulation. This indicates that mGluR functioned in the pre-synapse. Concerning the mechanism of $Ca^{2+}$ release from intracellular stores dependent on mGluR, the glutamate released by highly frequent stimulation activates PLC (phospholipase C) via mGluR and PLC enzymatically breaks down membrane phospholipids to form DAG (diacylglycerol) and $IP_3$ (inositol 1,4,5-trisphosphate). DAG modulates channel activity through PKC (protein kinase C) and $IP_3$ mobilizes the increase of $[Ca^{+2}]i$ from intracellular stores. The increase in $[Ca^{+2}]i$ does not occur as quickly as the opening of VGCCs. Finally, PKC via mGluR and CaM kinase via NMDAR seem to play important roles in the maintenance of LTP. Additionally, CREB phosphorylated by PKA (protein kinase A) and MAP kinase is also important to maintain LTP (2). However, there is no unified view regarding the molecular mechanisms of LTP's induction, while numerous works at molecular level have been reported. Therefore, further research into these molecular mechanisms in addition to the involvement of carbohydrates is needed.

Some forms of LTD appear to be mediated by the NMDAR and the VGCCs and seem to result from depotentiation. It was suggested that a low $[Ca^{+2}]i$ activates a protein phosphatase and then causes LTD, while a high $[Ca^{+2}]i$ causes LTP (21). A recent report suggested that inhibition of VGCCs in presynapses induces LTD in interneuronal synapses in the mossy fiber-stratum lucidum (21). Finally, progress in the molecular study of LTD is also expected in the future.

### C. Polysialic Acid

PSA (Polysialic acid) is a post-translational modification consisting of a homopolymer of α2,8-linked sialic acids present in the cell membrane. In the brain, PSA is found in a limited number of glycoproteins and predominantly on the neural cell adhesion molecule (NCAM) and sodium channel α subunit (22,23). While research has so far been more frequent concerning PSA-NCAM, concerning the PSA

後の細胞内カスケードにより、LTPが生じると考えられている。一方で、このようなNMDARを介した$Ca^{+2}$流入により誘導されるLTP (NMDAR-dependent LTP) とは異なる、NMDARを介さないLTPの誘導も存在する (15–17)。例えば苔状繊維-CA3錐体細胞間シナプスは、NMDAR阻害剤であるAPV(2-amino-5-phosphonovalerate)存在下で、LTPを発現する。この時、APVと共にVGCC阻害剤を与えることにより、LTPが消失することから、NMDARの代わりにVGCCが、LTPの誘導に関与することがわかる (15、16、18)。NMDAR-dependent LTPが多くを占めるCA3-CA1シナプスにおいても、一部VGCCが、LTPの誘導に関与することが知られている。

加えて，代謝型グルタミン酸受容体 (mGluR, metabotropic glutamate receptor) も細胞内カルシウム濃度の上昇に関与することが知られている (19)。代謝型グルタミン酸受容体は，後シナプスだけでなく前シナプスにも存在する。例えばCA3-CA1シナプスにおいて、mGluRの拮抗剤であるMCPG [(+)- α -methyl-4-carboxy-phenylglycine] を投与した後、高頻度刺激 (high-frequency stuimulation) を与えると、LTPの誘導が抑制される。その一方で、高頻度刺激を与えた後にMCPGを投与した場合は、LTPの誘導がおこる。これは、mGluRが前シナプスにおいて作用したことを示している (19)。mGluRのカルシウム放出に関わる分子メカニズムについては、高頻度刺激により放出されたグルタミン酸が、mGluRを介してPLC (phospholipase C) を活性化し，PLCが膜上のリン脂質をDAG (diacylglycerol) と$IP_3$ (inositol 1,4,5-trisphosphate) に分解後，DAGがPKC (protein kinase C) を介してチャネルを活性化し、$IP_3$が細胞内カルシウム濃度の亢進をになうようである。mGluRを介したカルシウムの放出は，カルシウムチャネルを介した反応に比べゆっくりしたものであることからも，mGluRを介したPKCは、先に述べたNMDARを介したCAM kinaseと共にLTPの維持に重要な役割を演じるようである。さらに、長期増強の維持に，protein kinase A (PKA), MAP kinaseによりリン酸化を受けるCREBの転写活性の亢進も必要であると考えられている (2)。しかしながらLTP誘導の分子機構は、いまだ多くの点で共通の見解が得られていない事が多く、糖鎖の関与と共に今後のさらなる研究の進展が期待される。

LTDもLTPと同様，NMDARやVGCCsの関与が示唆されているが，細胞内カルシウム濃度のちがいにより，その後のシグナル系の変化がLTDの誘導に関与しているようである (20)。最近の論文では、苔状繊維 - 透明層 (stratum lucidum) における介在ニューロン (interneuron) シナプスでは、前シナプスの電位依存性$Ca^{+2}$チャネルの抑制が、LTDを誘導する事例が報告されており (21)、LTDの分子機構についても、今後の研究の進展が期待される。

### C. ポリシアル酸 (PSA)

ポリシアル酸は、α -2,8結合したシアル酸の直鎖状ポリマーからなる細胞表面糖鎖であり、脳でのポリシアル酸修飾を受ける担体糖タンパク質として、神経細胞接着分子 (NCAM) と膜電位依存性ナトリウムチャネルαサブユニットが知られている (22、23)。PSA-NCAMが、最もシナプス可塑性について研究が進んでいる。その一方で、ナトリウムチャネルαサブ

in the sodium channel α subunit, researches have focused on the involvement of sialic acids with channel activity (24). Therefore, this review mainly describes PSA-NCAM.

NCAM is expressed on the membranes of neurons and glia cells and is an adhesion molecule that promotes cell-cell interaction with homophilic binding. NCAM is classified by its type of cytoplasmic domain into NCAM-120 [glycosylphosphatidylinositol (GPI) anchor type], NCAM-140 [short cytoplasmic domain], and NCAM-180 [long cytoplasmic domain]. Only NCAM-180 contains PSA. Two related enzymes: ST8Sia II (STX) and ST8Sia IV (PST), are responsible for the polymerization of sialic acid in α2 → 8 linkages in the fifth Ig (immunoglobulin)-like domain of the extracellular domain (25–27). *In vitro* analysis has suggested that ST8Sia IV has greater polymerizing activity than ST8Sia II and the coexistence of these enzymes increases the activity (28).

In polysialylated-NCAM, ionic repulsion caused by the cationic charge of polysialic acid on NCAM is an impediment to membrane-membrane contact (29) and increases elasticity structurally, leading to an increase of neural plasticity. Concrete examples include the outgrowth of neurites and axons and structural changes of synapses (30–34). Furthermore, several experimental approaches: treatment with Endo-N (endoneuraminidase) and mice deficient in ST8Sia II, ST8Sia IV, and NCAM, have revealed roles of PSA-NCAM in synaptic plasticity. Therefore, this review focuses on the function of PSA in synaptic plasticity.

First, it explains the distribution of PSA and NCAM in the hippocampus according to the unidirectional connection described in "Mechanisms of hippocampal LTP" (Fig. 1). Immunoelectron microscopy with anti-NCAM-180 antibody demonstrated that about one-third of the postsynaptic density expresses PSA-NCAM in the molecular layer of the dentate gyrus receiving the perforant pathway (35). The ratio of one-third obtained with anti-NCAM-180 antibody is consistent with that obtained with anti-PSA antibody (36,37). On the other hand, PSA and NCAM also seem to occur in the presynaptic membrane independently. While NCAM is expressed in the mossy fiber bundles and boutons, PSA is distributed in unmyelinated axons with the mossy fiber bundles and immature boutons. These results show that not all NCAM is modified with PSA (33, 38). Additionally, there was no expression of either NCAM or PSA in the DG-CA3 synapse receiving the mossy fiber (36). Finally, axons on the Schaffer collateral and pre- and post-synaptic membranes in the CA1 pyramidal layer are PSA-positive, which seems to show PSA-NCAM-positivity, because the PSA in the Schaffer collateral disappears in NCAM-deficient mice (39). Referring to this distribution, the involvement of PSA-NCAM in synaptic plasticity is described below.

ユニットにおける、シアル酸修飾とチャネル活性の関連性についての研究は、骨格筋あるいは心筋のナトリウムチャネルに注目した研究が中心であることから(24)、本稿ではPSA-NCAMについて解説する。NCAMは、ニューロンやグリア細胞膜上で発現し、そのホモフィリックな結合により細胞間相互作用を促進する、細胞接着因子である。NCAMは、細胞質ドメインのちがいにより、NCAM-120[GPI(glycosylphosphatidylinositol)アンカータイプ], NCAM-140 [short cytoplasmic domain], NCAM-180 [long cytoplasmic domain]に分けられ、NCAM-180のみ、ポリシアル酸化を受ける。これは，NCAM細胞外ドメイン上の第5Ig (immunoglobulin)様ドメインにあらかじめ付加されたN結合型糖鎖末端のシアル酸が，ST8Sia II (STX)とST8Sia IV (PST)により，α 2→8結合シアル酸重合を受けるものである(25–27)。これら2種の酵素活性については、共に直鎖状のポリシアル酸にシアル酸を転移し、ST8Sia IIよりもST8Sia IVの方が重合活性が高く、さらにST8Sia IIとST8Sia IV共存時が最も重合活性の高いことが*in vitro*の実験系より証明されている(28)。

ポリシアル酸化されたNCAM-180は、ポリシアル酸の負電荷による反発により、NCAM本来の細胞間相互作用の能力を消失し(29)、構造学的に融通性を亢進し、可塑性を高めるようである。具体的には，神経発生の時期における突起伸展や軸索の伸長、さらにはシナプスの構造変化を示す報告がある(30–34)。さらに、PSAを分解するEndo-N(endoneuraminidase)を利用した研究に加え、NCAM, ST8Sia IIそしてST8Sia IVのノックアウトマウスが作成されたことから、シナプス可塑性へのPSAとNCAMの役割がずいぶん明らかになってきた。

まずは、海馬内における発現分布を、「海馬LTP発生機序」の項で記した海馬内繊維連絡に従って記す(図1)。まずPSA-NCAMは、貫通繊維路が投入される海馬歯状回分子層に分布する後シナプス肥厚のおおよそ1/3に発現している(35)。この1/3という比率は、抗PSA抗体(735 mAb, MenB mAb)及び抗NCAM-180抗体を用いた免疫電顕により同様の結果を得ていることから、PSA-NCAMの存在を表していると考えられる(36,37)。一方で、PSAとNCAMそれぞれは、後シナプス肥厚だけでなく一部の前シナプス膜にも存在することから、前シナプス膜におけるPSAの担体については疑問が残る。次に、苔状繊維においては、NCAMが、苔状繊維束及び投射先であるCA3領域内の神経終末に分布する。その一方でPSAは、苔状繊維束のうちミエリン梢を巻いていない軸索と、未分化な一部の神経終末に分布しており、すべてのNCAMがPSA-NCAMではないことを示している(33,38)。さらに、苔状繊維の投射によるDG-CA3シナプスにはNCAM及びPSAは共に存在しないようである(36)。最後に、シャファー側枝については、シャファー側枝に当たる軸索及びCA1領域に存在するCA3-CA1シナプスの前シナプス膜及び後シナプス膜共にPSA陽性であった。このシャファー側枝におけるPSA分布は、NCAM欠失ノックアウトマウスでは陰性であったことから、PSA-NCAMの分布を示していると考えられる(39)。以上のPSA-NCAMの分布を参考に、以下にPSAとシナプス可塑

**Table. I. Roles of polysialic acid and NCAM in neural development and synaptic plasticity.** This table was modified from two tables in Ref 42,43, and 44. – indicates impairment of function; + indicates normal function. n.d., not detected; n.d.*, there might be no difference; $ shows that impairment was rescued by elevation of extracellular $Ca^{2+}$ concentrations from 1.5 to 2.5 mM; # shows impairment in the adult but not juvenile.

| Function | Endo-N | NCAM-/- | conditional NCAM-/- | ST8Sia IV-/- | ST8Sia II-/- | Molecular basis |
|---|---|---|---|---|---|---|
| Migration of neural precursors | –[43,50] | –[43,51,52] | n.d.* | +[43] | +[42] | PSA-dependent NCAM function |
| Lamination of mossy fibers | –[33] | –[41] | +[44] | +[43] | –[42] | ST8Sia II-dependent PSA-NCAM function |
| STP in CA3-CA1 synapse | –[34,39] | –[39] | +[44] | +[43] | +[42] | PSA-dependent NCAM function |
| STP in DG-CA3 synapse | +[42] | +[41] | +[44] | +[43] | +[42] | No effect of PSA or NCAM |
| STP in EC-DG synapse | n.d. | –[40] | n.d. | +[40] | +[40] | PSA-independent NCAM function |
| LTP in CA3-CA1 synapse | –[34,39] | –[39] | –$,[44] | –#,[43] | +[42] | ST8Sia IV-dependent PSA-NCAM function |
| LTP in DG-CA3 synapse | +[42] | –[41] | +[44] | +[43] | +[42] | PSA-independent NCAM function |
| LTP in EC-DG synapse | n.d. | –[40] | n.d. | +[40] | +[40] | PSA-independent NCAM function |
| LTD in CA3-CA1 synapse | –[39] | n.d. | –$,[44] | –#,[43] | n.d. | ST8Sia IV-dependent PSA-NCAM function |

In 1996, Muller *et al.* indicated that the treatment of hippocampal slices with Endo-N resulted in the disappearance of LTP or LTD in the CA3-CA1 synapse (39). Then, several reports were published about analyses of PSA-NCAM in mice deficient in ST8Sia II, ST8Sia IV, and NCAM (Table I). In Table 1, STP shows an initial response following high frequency stimulation and LTP shows a long-lasting response for more than several minutes following stimulation. NCAM is involved in most of the synaptic plasticity of the three pathways of the hippocampus in Table 1, except for no response of STP in the DG-CA3 synapse (39–42).

First, LTP in the perforant pathway requires NCAM but not PSA (40). The expression of PSA-NCAM in one-third of postsynaptic density in the perforant pathway (35) has led to postulation of the involvement of PSA-NCAM in LTP and we expect progress in further investigations. Second, while PSA expressed depending on ST8Sia II is necessary for lamination of the mossy fiber, the STP and LTP in mossy fibers is PSA-independent (41,42), which might result from no expression of PSA in DG-CA3 synapses. Finally, LTP and LTD in the Schaffer collateral require PSA-NCAM (39, 42-45), which is not incongruous with the expression patterns of PSA-NCAM in pre- and post-synaptic membranes of CA3-CA1 synapses. Additionally, conditional ablation of NCAM by cre-recombinase under the control of the CAM kinase II promoter resulted in inhibition of LTP and LTD in the CA3-CA1 synapse (44). The ablation resulted from the recombinase activity in neurons involved in NMDA-dependent LTP, suggesting a correlation between PSA and NMDAR in the CA3-CA1 synapse. NMDAR consists of NR1 and NR2 subunits which are expressed in the pyramidal cells of the CA1 subfield. Recently, it has been reported that PSA-NCAM affected the NR2B subunit, that is, soluble PSA and PSA-NCAM inhibited single NMDAR channel activity stimulated by glutamate (46). The inhibition by PSA and PSA-NCAM was fully occluded by the NR2B-specific antagonist,

1996年にMullerらは(39)、海馬スライスにEndo-N処理を行い、CA3-CA1シナプスにおけるLTPとLTDの消失を示した。このMullerらの報告の後、Endo-N処理に加えて、NCAM, ST8Sia II, ST8Sia IVのノックアウトマウス海馬における解析が行われてきた（表1)。表1におけるSTPとは、高頻度刺激後の初速時の反応を示し、LTPはその後数十分間にわたる長期増強を示している。NCAMは、苔状繊維におけるDG-CA3シナプスのSTPに関与しないことを除けば、海馬における3神経連絡のほとんどすべてのシナプス可塑性に関与している(39–42)。

貫通繊維路に関するLTPについては、NCAMは必須であるがPSAの影響を全く受けない。EC-DGシナプスの1/3がPSA-NCAMであることから(35)、PSAのシナプス可塑性への何らかの関与が示唆され、LTDを含めた今後の解析が待たれる(40)。次に苔状繊維の束化にはPSAの発現が関与し、特にST8Sia IIの発現が必須である(41,42)。その一方で、苔状繊維のLTPについては、PSAの影響を受けない。これは、DG-CA3シナプスにPSAの発現がないことから、シナプス可塑性には直接影響しないのかもしれない。さらにNCAMが、DG-CA3シナプスのSTPに作用しないことも、シナプスに発現していないことに関連するのかもしれない。最後に、シャファー側枝におけるシナプス可塑性は、PSA-NCAMのLTPやLTDへの影響をもっともよく説明している(39,42–45)。LTP及びLTD共に、PSA-NCAMの影響を強く受ける。これは、CA3-CA1シナプスの前シナプス膜及び後シナプス膜共にPSA-NCAMが分布していることからもシナプス可塑性への関与を裏付けることができる。また、NMDAR-dependent LTPの維持に重要であると考えられているCaM kinase IIのプロモーター支配下のCre遺伝子を用いた、コンディショナルNCAM欠失マウス海馬においても、CA3-CA1シナプスにおけるLTPとLTDが阻害された。こうした阻害は、NMDAR-dependent LTPに関与するニューロンにのみNCAMを欠失させた結果であることから、CA3-CA1シナプスにおけるNCAMとNMDARとの関連性を示唆することになる（44)。NMDARは、NR1とNR2サブユニットからなり、CA1錐体細胞には、NR2AとNR2Bの存在が知られているが、最近の知見から、PSA-NCAM

ifenprodil. These observations indicate that PSA and PSA-NCAM interact with the NR2B containing NMDA receptors. However, in the hippocampus, synaptic NMDA receptors contain NR2A and extrasynaptic NMDA receptors contain NR2B (47), which causes loss of mitochondrial membrane potential (an early marker for glutamate-induced neuronal damage) and cell death. These results raise doubt about whether the effect of PSA-NCAM on NR2B is involved in synaptic plasticity. On the other hand, a report found that post-synapses contain NR2B, which affects the induction of LTP in the amygdala which receives projections from the hippocampus (48). Therefore, also in the hippocampus, the effect of PSA-NCAM on NR2B may be directly involved in NMDAR-dependent LTP.

PSA expressed dependent on ST8Sia IV is necessary for the induction of LTP and LTD in the CA3-CA1 synapse of only adult mice (more than 4 weeks old) (43). The abundant expression of ST8Sia II in newborn mouse brain shifts toward a prominent expression of ST8Sia IV in the adult brain (49). This expressional shift might affect differences between juveniles and adults concerning the acquirement of synaptic plasticity.

Furthermore, previous reports have shown the involvement of PSA-NCAM in higher neural plasticity. In a fear-conditioning test suitable for observations of emotional stress (45), the injection of PSA-NCAM-Fc into the hippocampus *in vivo* resulted in a reduction of freezing time when the mice entered a room where they had previously received footshocks (contextual learning). On the other hand, NCAM-deficient mice have a disturbed contextual memory. The mice received an injection of PSA-NCAM-Fc or NCAM-Fc into the hippocampus, resulting in an extension of or no effect on the freezing time, respectively. As these effects of PSA-NCAM do not occur following an injection into the amygdala, an adequate dose of PSA in the hippocampus seems to be involved in emotional stress. The suprachiasmatic nuclei (SCN) of the hypothalamus contain an endogenous circadian clock that maintains synchrony with the external environment through light input and express PSA-NCAM. In rat brain slices recorded for 3 days, the mean firing frequency of SCN neurons was recorded as a sinusoidal curve over a 24 hour period with a peak at midday, resembling the circadian rhythm, and treatment of the slices with glutamate produced light-like shift of 3 hours in the circadian rhythm (54). On the other hand, in NCAM-180-deficient mice, circadian rhythmicity was abolished, resulting from a determination based on activity in darkness (55). Furthermore, treatment with Endo-N in the SCN disturbs the light-like shift caused by glutamate (53). These results suggest that PSA affects circadian rhythm and is also relevant to synaptic plasticity via glutamate stimulation. Additionally, several reports have

がNR2Bに作用することがわかってきた(46)。細胞外に添加したPSAやPSA-NCAMがグルタミン酸刺激による単一のNMDARチャネル活性を阻害するものである。またNR2B特異的阻害剤（ifenprodil）が、PSAやPSA-NCAMによるチャネル活性阻害と競合することから、PSAやPSA-NCAMが、NR2Bに作用することが明らかとなった。しかしながら、海馬ニューロンのシナプスNMDARがNR2Aであり、シナプス外NMDARがNR2Bであるという報告があること(47)、さらには、NR2B阻害がグルタミン酸刺激による細胞死を抑制することから、NR2Bを介したPSA-NCAMの作用がシナプス可塑性に直接関与するかどうかは、議論の残すところがある。しかしながら最近、海馬に入力繊維を持つ扁桃体において、NR2Bがスパイン（後シナプス）に存在し、LTPに関与する知見が報告された(48)。それ故、CA1シナプスにおいてもNR2Bを介したNMDAR-dependent LTPが存在し、PSAやPSA-NCAMが関与している可能性も残されている。

加えて、CA3-CA1シナプスにおけるLTP及びLTDに関わるPSAは、ST8Sia IVによるポリシアル酸転移によるようである(43)。ただしそれは4週齢以降のマウスにおいて観察された結果で、2週齢のST8Sia IV欠失マウスでは影響がない。これは、ST8Sia IIが発生期に強く発現し、出生後成熟するにつれてST8Sia IVの発現に移行することに反映されていると思われる(49)。すなわち、シナプス可塑性の獲得方法が幼弱期と成熟期の間で異なり、PSAの関わり方も変化すると考えられる。

さらにPSA-NCAMについては、高次の神経可塑性への関与を示唆する報告がある。例えば、情動ストレスの観察に適した恐怖条件付けストレスを与えたマウスの海馬内へ、PSA-NCAM-Fcを注入した後、電気ショックを受けた部屋に再び入れるとfreezing時間が短縮する。その一方で、NCAM欠失マウスはもともとfreezinig時間が短く、海馬にPSA-NCAM-Fcを注入するとfreezing時間が延長するが、NCAM-Fcでは効果がない。これは海馬におけるPSA量の調整が、情動ストレスに関与していることを示している。さらに扁桃体への注入ではfreezinig時間に影響を与えないことから、海馬に特異的なシナプス可塑性の高次機能を示していると考えられる(45)。またPSAは、サーカディアンリズムの獲得にも寄与していることが知られている(53)。視床下部視交叉上核(SCN)が、外界からの明暗信号を受け取ることによりサーカディアンリズムの同調が生じる。In vitro SCNスライスに記録電極を入れると、発火周波数が正弦曲線を描く明期にあたる時間（明期様）と、正弦曲線を描かない暗期にあたる時間（暗期様）からなる、24時間の日周リズムが得られる（3日間生存時のリズムをきざむ）。そして暗期にグルタミン酸を投与すると、明期に見られる周波数のピークが3時間移相する事が知られている（54）。NCAM-180欠失マウスでは、明暗期における運動活性のリズムが同調しなくなること（明期は睡眠、暗期は活動）が明らかになり(55)、さらにはSCNスライスにおけるグルタミン酸刺激による周波数ピークの3時間移相が、Endo-N処理により阻害されることから、PSAの発現がSCNのサーカディアンリズムに関与し、グルタミン酸を介するシナプス可塑性との関

proposed effects of PSA on neural plasticity via neuronal hormones: lactation by stimulation with oxytocin in supraoptic nuclei of the hypothalamus (56), estradiol-induced increase in the number of GABAergic axo-somatic synapses in the arcuate nucleus of the hypothalamus (57), and developmental migration of neurons expressing luteinizing hormone-releasing hormone (LHRH) (58) and so on. Other reports have also suggested that PSA has relevance to neuronal diseases including epilepsy (59, 60), heroin addiction (61), and the proliferation of neural stem cells (62).

**D. HNK-1**

The carbohydrate HNK-1 is carried by glycolipids and glycoproteins including the immunoglobulin (Ig) superfamily (NCAM, P0, L1, and F3/F11/contactin), integrin, proteoglycans, and the extracellular matrix glycoproteins (tenascin-C, -R). HNK-1 is involved in neural development and synaptic plasticity through the mediation of cell-cell recognition. HNK-1 is a sulfated trisaccharide ($HSO_3$-3GlcAβ1-3Galβ1-4GlcNAc) and two glucuronyltransferases (GlcAT-P, GlcAT-S) and one sulfotransferase (HNK-1ST) are involved in its biosynthesis. Studies have been conducted with GlcAT-P and HNK-1ST deficient mice to know whether HNK-1 is involved in neural plasticity (63,64). Deficiencies of both GlcAT-P and HNK-1ST resulted in an inhibition of LTP in the CA3-CA1 synapse, suggesting the involvement of HNK-1 in synaptic plasticity. The molecular mechanism of LTP via HNK-1 needs to be analyzed in more detail. On the other hand, it has been reported that the application of anti-HNK-1 antibody induced an increase of LTP and decrease of $GABA_A$ receptor-mediated pIPSCc (perisomatic inhibitory postsynaptic currents) in CA3-CA1 synapses (65). HNK-1 antibody did not affect pIPSCs in knock-out mice deficient in tenascin-R, but did affect them in NCAM-deficient mice. These results provide evidence that HNK-1 carried by tenascin-R is involved in channel permeability of $GABA_A$ receptor at least in some hippocampal neurons.

**E. Fucα(1-2)Gal**

Fucose-α(1-2)-galactose [Fucα(1-2)Gal], which exists as a terminal carbohydrate modification to N- and O-linked glycoproteins, has been implicated in learning and memory (66,67). 2-deoxy-D-galactose (2-dGal) prevents the formation of Fucα(1-2)Gal linkages by incorporation of the drug into glycan chains, reversibly. On injection of 2-dGal into the rat intra vein, STP and LTP which should be induced by high frequency stimulation are interfered with in the EC-DG synapse (perforant pathway) and CA3-CA1 synapse (Schaffer collateral) (68). Also, the injection of an antibody (A46-B/B10) recognizing the Fucα(1-2)Gal epitope impaired the retention performance of rats in a relearning session with

連性が示唆される (53)。この他 PSA は、オキシトシン刺激による泌乳刺激時における視床下部視索上核 (SON) における反応 (56)、エストロゲン刺激による抑制性ニューロンシナプス数の制御 (57)、さらには、黄体形成ホルモン放出ホルモン発現ニューロンの発生時の遊走 (58) 等、神経ホルモンに関わる可塑的変化への関与を示唆する報告がある。またてんかん形成 (59、60) やヘロイン中毒 (61) 等の疾患や、神経幹細胞の増殖 (62) との関連性の可能性を示唆する論文も報告されており、今後のさらなる研究の進展が望まれる。

D. HNK-1

HNK-1 糖鎖は、糖脂質あるいは、イッムノグロブリン (Ig) スーパーファミリー (NCAM, P0, L1, F3/F11/contactin) やインテグリン, プロテオグリカン, 細胞外マトリクス tenascin-C, tenascin-R 等の糖タンパク質に付加する糖鎖で、細胞間認識機構を利用した神経発生やシナプス可塑性に関与する。この HNK-1 は、N- アセチルラクトサミン構造の非還元末端に存在するガラクトース残基にグルクロン酸を転移する 2 種のグルクロン酸転移酵素 (GlcAT-P, GlcAT-S) と硫酸基を転移する硫酸基転移酵素 (HNK-1ST) より合成され、近年 GlcAT-P と HNK-1ST のノックアウトマウスが作成され、シナプス可塑性について調べられている。共に CA3-CA1 シナプスにおける LTP の抑制が観察され、HNK-1 のシナプス可塑性への関与が示唆された (63、64)。この LTP への関与に関する分子メカニズムは今後の解析を待つところである。しかしながら、HNK-1 抗体が、CA3-CA1 シナプスの LTP を上昇させると共に、$GABA_A$ 受容体由来の pIPSC の振幅を低下させた報告がある (65)。この pIPSC は、NCAM 欠失マウス海馬でも、野生型と同様に観察されたが、tenascin-R 欠失マウス海馬ではおこらなかった。これは、tenascin-R に付加された HNK-1 が、少なくとも一部の海馬ニューロンの $GABA_A$ 受容体のチャネル透過性に関わっている事を示している。

E. Fuc α (1-2)Gal

Fuc α (1-2)Gal は、N- 結合型、O- 結合型糖タンパク質の糖鎖の末端に付加され、学習記憶に関わることが以前より示唆されてきた (66、67)。2-deoxy-D-galactose (2-dGal) は、脳での Fuc α (1-2)Gal 残基形成を阻害する試薬である。例えばあらかじめ電極を挿入したラットの尾静脈に 20 μ mol 2-dGal 注入 30 分後、EC-DG シナプス (貫通繊維路) 及び CA3-CA1 シナプス (シャファー側枝) に高頻度刺激をおこなったところ、STP の抑制が観察され、その後の追加 (20 μ mol) により、LTP の誘導も抑制された (68)。また Fuc α (1-2)Gal エピトープを認識する抗体 (A46-B/B10) を海馬内に注入後、情動ストレス学習 (明暗箱のうち暗所に入るとフットショックが誘導される passive

passive avoidance, which is a method of determining the effect on learning of an unpleasant stimulus [When rat enters a dark alley, rat is punished by footshocks despite preference for dark](69). Many reports implicate Fucα(1-2)Gal in amnesia with behavioral analyses.

Finally, it was recently clarified that the carrier of Fucα(1-2)Gal is synapsin Ia and Ib (70). The A46-B/B10 antibody recognizes synapsin. The addition of 2-dGal to cells prevents the fucosylation of synapsin, resulting in synapsin's degradation mediated by the calcium-dependent protease calpain. The de-fucosylation changes the cellular half-life of synapsin from 18 to 5.5 hours.

Synapsin tethers synaptic vesicles to the actin cytoskeleton. An increase of $[Ca^{2+}]i$ activates PKA (protein kinase A) and CaM kinase, resulting in phosphorylation of synapsin. Then, phosphorylated synapsin withdraws its role as a tether and synaptic vesicles prepare to dock and fuse with the active zone of the presynaptic membrane, resulting in the release of neurotransmitters. Therefore, a steady supply of synapsin is very important to the acquirement of synaptic plasticity and Fucα(1-2)Gal bears the primary responsibility for the stability of synapsin.

**F. Proteoglycan**

Proteoglycans are heavily *O*-glycosylated proteins. The carbohydrate structure is called glycosaminoglycan (GAG) and composed of repeating disaccharide units: amino sugar derivatives and hexose derivatives, with diversity in the position and quantity of sulfation. Proteoglycans are secreted into the extracellular matrix and inserted into the plasma membrane, and influence cell-environment interactions by binding to a heterogeneous group of growth factors and other matrix ligands, and adhesion molecules. In the central nervous system, hyaluronan, chondroitin sulfate, and heparan sulfate among proteoglycans, especially, influence the development of neural circuits and regeneration of neural injury (71–73). Furthermore, the involvement of chondroitin sulfate and heparan sulfate in synaptic plasticity has been reported.

**F-1. Chondroitin Sulfate**

Treatment with chondroitinase ABC of hippocampal slices resulted in the disappearance of LTP or LTD in the CA3-CA1 synapse (Schaffer collateral) (74). On the other hand, it has been reported that tenascin-R binds with chondroitin sulfate and the CA3-CA1 synapse of tenascin-R-deficient mice exhibits a reduction of LTP compared to the wild type (75). The degree of the reduction is the same with or without chondroitinase ABC, suggesting that binding between tenascin-R and chondroitin sulfate is involved in the development of LTP. On the other hand, there is no effect in tenascin-R-deficient mice on LTD in the CA3-CA1 synapse, showing that the effect of chondroitinase ABC on LTD is

avoidance を用いており、ラットは暗所を好むことから、不快刺激の学習効果をみるもの。）を行ったところ、学習効果が減弱したことから、海馬依存の空間認知記憶が傷害されていることがわかった (69)。以上のことから、Fuc α (1-2)Gal は健忘症関連糖鎖であると認知されていた。

最近になって、Fuc α (1-2)Gal の主要担体が synapsin Ia と Ib であることが判明した (70)。その発端は先に用いた A46-B/B10 抗体が、シナプシンを認識したことにある。そして、シナプシンは 2-dGal を取り込むことにより、Fuc α (1-2)Gal 残基を持たなくなり、その結果、カルパイン (calcium-dependent protease calpain) に分解されるようになり、Fuc α (1-2)Gal を持たないシナプシンの半減期は、18 時間から 5.5 時間へと減少する。

シナプス小胞は、シナプシンを介してアクチン繊維につなぎ止められており、シナプス前終末の脱分極により細胞内に $Ca^{2+}$ が流入することで、PKA や CaM kinase が活性化され、シナプシンはリン酸化される。シナプシンがリン酸化されるとシナプス小胞はアクチン繊維から遊離し、シナプス前膜のアクティブゾーンにドッキングし、神経伝達物質の放出を待つ。このことから、シナプシンの安定供給は、シナプス可塑性の獲得にとって非常に重要であることがわかり、Fuc α (1-2)Gal 修飾が、シナプシンの安定化を担っていることが明らかとなった。

F. プロテオグリカン

プロテオグリカンは O- 結合型糖タンパク質で、糖鎖部分はグリコサミノグリカンと呼ばれ、アミノ糖とウロン酸の 2 糖単位の繰り返し構造からなり、硫酸基の修飾の位置と程度により多様性を持つ。プロテオグリカンの多くは、細胞外マトリクスに含まれ、他の細胞外マトリクスや細胞接着因子と結合し、細胞間コミュニケーションや、細胞間の空間維持に関与している。中枢神経系では、これまで脳の神経回路発達や中枢神経損傷後の修復に、ヒアルロン酸、コンドロイチン硫酸、ヘパラン硫酸の関与が明らかになってきている (71–73)。さらにプロテオグリカンの中でも、特にコンドロイチン硫酸、ヘパラン硫酸がシナプス可塑性に関与することが知られており、本稿では、このシナプス可塑性への関与について解説する。

F-1. コンドロイチン硫酸

海馬をコンドロイチナーゼ ABC 処理によりコンドロイチン硫酸を分解した後、CA3-CA1 シナプス（シャファー側枝）に高頻度刺激をおこなったところ、LTP 及び LTD が抑制された (74)。一方、以前より LTP に関与する細胞外マトリクスでコンドロイチン硫酸に結合する分子が tenascin-R であることが知られていた (75) ことから、tenascin-R 欠失マウス海馬への ABC 処理の効果を見たところ、ABC 処理前後 LTP 抑制効果は同じであった。この結果は、少なくともコンドロイチン硫酸の tenascin-R との結合が LTP の発生に関与していることを示唆している。一方で、tenascin-R 欠失マウス海馬は LTD の影響がみられないことから、ABC 処理による LTD 効果は、

induced by the binding of chondroitin sulfate to a component of the extracellular matrix other than tenascin-R. Furthermore, mice deficient in Ptprz (protein tyrosine phosphatase receptor type Z), having domains for binding with chondroitin sulfate, also showed a reduction of LTP compared to wild-type mice in the CA3-CA1 synapse (76). These results suggest that chondroitin sulfate binds with Ptprz, tenascin-R, HNK-1, and so on extracellularly and seems to coordinate synaptic plasticity.

The involvement of brevican and NG2, proteoglycans containing chondroitin sulfate, in synaptic plasticity has been reported. First, brevican-deficient mice showed significant deficits in the maintenance of LTP in the CA3-CA1 synapses of hippocampal slices. Additionally, application of anti-brevican antibody also shows deficits in the LTP in the CA3-CA1 synapse (77). Alternatively, NG2 is expressed in 5 to 10 % of all cells, depending on the brain region, especially in the hippocampus. NG2-positive cells are glia that do not express GFAP (glial fibrillary acidic protein) or MBP (myelin basic protein). NG2-positive cells are not astrocytes or oligodendrocytes. Furthermore, the neuron-glia synapse is found in NG2-positive cells (78). Ge *et al.* made whole-cell recordings from NG2 cells in the CA1 region of rat hippocampal slices by inducing glial cell membrane currents with a high frequency stimulation of Schaffer collaterals (79). The identity of astrocytes and NG2 cells was determined by post-immunostaining. Theta burst stimulation resulted in a persistent increase in the EPSC (excitatory postsynaptic current) amplitude in NG2 cells, analogous to LTP found in synaptic plasticity existing between neurons. The LTP-like EPSCs were reduced by philanthotoxin-33, a toxin that specifically blocks the CaPARs ($Ca^{2+}$-permeable AMPA receptors), Kyn (NMDA, quisqualate, and kainite receptor blocker), and BAPTA (calcium cannel chelator). These results show that NG2 cells induce a NMDAR-independent LTP that depends on $[Ca^{2+}]i$. Further research is needed to know whether NG2 itself is implicated in synaptic plasticity, however, the involvement of NG2-positive cells in synaptic plasticity has been clarified.

**F-2. Heparan Sulfate**

Among proteoglycans with heparan sulfate, syndecan-3 is expressed in pyramidal cells in the CA1 subfield of the hippocampus and biological analyses show that FGF and HB-GAM bind syndecan-3 in the hippocampus. Two approaches have been used to investigate the effects of heparan sulfate on the induction and maintenance of LTP. First, treatment with heparitinase of hippocampal slices resulted in the disappearance of LTP in the CA3-CA1 synapse (Schaffer collateral) (34,80). Second, the application of a soluble syndecan-3 to hippocampal slices prevented LTP in the CA3-CA1 synapse. On the other hand, syndecan-3-deficient mice

tenascin-R 以外の細胞外マトリクスとの相互作用が影響していると思われる。また一方で、コンドロイチン硫酸接着領域を持つ Ptprz(protein tyrosine posphatase receptor type Z) の欠失マウス成熟海馬スライスの CA3-CA1 シナプス（シャファー側枝）に高頻度刺激をおこなったところ、LTP が抑制され、これも NMDA-independent LTP であった (76)。以上の結果より、コンドロイチン硫酸は、Ptprz, tenascin-R、HNK-1 などと共に細胞外で結合し合い、シナプス可塑性を調整しているのであろう。

一方で、コンドロイチン硫酸を持つプロテオグリカンの中で、brevican と NG2 に関するシナプス可塑性が報告されている。まず、brevican は直接 LTP に関与しているようである (77)。Brevican 欠失マウス海馬スライスは CA3-CA1 シナプス（シャファー側枝）における LTP の誘導が抑制され、さらには、コントロールマウス海馬スライスに抗 brevican 抗体を投与したところ、LTP の誘導が阻害された。一方で、NG2 に関する知見は、少し趣を異にしている。NG2 は、脳の 5-10% の細胞が発現しているが特に海馬に多く、GFAP (glial fibrillary acidic protein) 陰性及び、MBP (myelin basic protein) 陰性のグリア細胞に発現している。すなわち、一般に言われているアストロサイトでもオリゴデンドロサイトでもない細胞であり、さらに NG2 細胞は、ニューロンとシナプスを作る (78)。Ge らは、海馬スライスのシャファー側枝に LTP 誘導刺激を送り、CA1 領域に存在する NG2 細胞の膜電位をパッチクランプにより測定した (79)。NG2 細胞であるかどうかは、パッチクランプ測定後に免疫染色をおこない、個々に同定している。LTP 導入刺激 (theta burst stimulation) により、NG2 細胞は、興奮性後シナプス電流 (EPSCs) の拡大を示した。この LTP 様の反応は、$Ca^{2+}$ 透過性 AMPA 受容体の阻害剤 (PhTx) で抑制され、さらには、Kyn(NMDA, quisqualate, and kainate receptor blocker) や、カルシウムチャネルキレート剤 (BAPTA) でも、抑制された。これらのことから、NG2 細胞は、non-NMDA 受容体依存的に LTP を誘導し、この LTP は、ニューロンーニューロン間シナプスと同様に細胞内 $Ca^{2+}$ 濃度に依存した LTP であることが明らかとなった。実際に NG2 自身が、LTP に関与しているかどうかは、今後の検討課題ではあるが、少なくとも、NG2 を発現しているグリア細胞が、LTP 誘導に関わっていることが明らかとなった。

F-2. ヘパラン硫酸

ヘパラン硫酸を持つプロテオグリカンのうち、シンデカン -3 が海馬 CA1 錐体細胞に発現することが知られている。さらに生化学的解析より、海馬ではシンデカン -3 が細胞外で FGF や HB-GAM と結合することがわかっている。ヘパラン硫酸についても、先に示してきた方法論によりシナプス可塑性への関与について調べた知見がある。まず、海馬をヘパリチナーゼ処理によりヘパラン硫酸を分解した後、CA3-CA1 シナプス（シャファー側枝）に高頻度刺激をおこなったところ、LTP が抑制された (34,80)。また可溶性シンデカン -3 を海馬 CA1 領域に添加後 CA3-CA1 シナプスに高頻度刺激をおこなうと、LTP が抑制された。一方で、シンデカン -3 欠失マウス海馬では、

showed an increase of LTP in the CA3-CA1 synapse (81). Previous reports have indicated that FGF increases LTP (82) and HB-GAM impairs LTP (83) in the hippocampus. The heparan sulfate might coordinate the effects of FGF and HB-GAM on neural plasticity. Recent research has indicated that *Drosophila* heparan sulfate proteoglycan: syndecan and Dallylike [glycosylphosphatidylinositol (GPI) anchored glypican] play important roles in the development of synapses in neuromuscular junctions (84). It is known that boutons in presynaptic terminals contain active zones that organize glutamate's release and an increase of synaptic activity enlarges the active zone per bouton, which requires LAR family RPTPs (protein tyrosine phosphatases) (85). First, both syndecan and dallylike bind LAR. Second, the syndecan-deficient mutant shows a decrease of bouton size and the Dallylike-deficient mutant shows a decrease of active zone size. Finally, a double mutant assay showed the requirement of LAR for actions of both syndecan and Dallylike. The discovery of the collaboration of these heparan sulfate proteoglycans in morphological synaptic plasticity will lead to analyses in mammalian systems including the mouse and rat.

## G. Concluding Remarks

This review focused on 5 species of carbohydrate, whose structure had been identified and for which there were knock-out mice deficient in the transferase required to synthesize the carbohydrate. There are reports that carbohydrates affect the maturation of channels, channel activity, and the transport of synaptotagmine to presynaptic terminals and so on (24,86–90) and that carbohydrates are implicated in synaptic plasticity (91–93). Furthermore, concerning the neural plasticity described in the introduction, analyses at the cellular and molecular level should progress in the near future and carbohydrates will attract more attention.

CA3-CA1 シナプスにおける LTP を亢進した (81)。以前より、海馬では FGF が LTP を亢進すること (82)、HB-GAM が LTP を抑制すること (83) が知られている。このことは、ヘパラン硫酸を介した FGF、HB-GAM、シンデカンの結合が、シナプス可塑性に何らかの関与を示していることを示唆する。最近2種のヘパラン硫酸プロテオグリカン；シンデカンと Dallylike [GPI (glycosylphosphatidylinositol ) anchored glypican] が、ショウジョウバエの神経筋接合部におけるシナプス発達機構に重要な役割を示すことが報告された (84)。シナプス前終末 (bouton) はグルタミン酸放出部分である active zone を含み、シナプス活性が亢進された場合、この bouton あたりの active zone が広がる。このシナプス形成にレセプター型チロシンホスファターゼの LAR ファミリーメンバーが関与することがすでに知られている (85)。その一方で、シンデカン欠失ミュータントは、bouton の大きさを縮小し、Dallylike 欠失ミュータントは、bouton あたりの active zone の縮小を示した。さらに、生化学的解析より、シンデカン及び Dallylike が共に LAR に結合すること、LAR 欠失ミュータントとのかけあわせにより、シンデカンは LAR を促進し、Dallylike は抑制することがわかった。以上のことから、ヘパラン硫酸プロテオグリカンがシナプス前終末におけるシナプス活性に重要な役割を持つことが明らかになった。今後マウスを含む哺乳動物におけるシナプス可塑性への生理学的な解析も進むと思われる。

## G. 結　語

本稿では、糖鎖が特定されているもの、ノックアウトマウスの存在あるいは分子間相互作用の知見が明らかになっているもの、に焦点を当てた。本稿で解説したような糖鎖の特定がなされていないものの中には、チャネルに付加された糖鎖が、チャネルの成熟や、チャネル活性の修飾に関与する例 (24,86–89) や、シナプス前終末へのシナプトタグミンの配置に糖鎖が関与する例 (90) 等、糖鎖がシナプス可塑性に関与する例が多く報告されている (91–93)。序論で述べたような複雑な神経可塑性に関しても、今後分子レベルに及ぶ研究が進み、糖鎖の重要性がさらに注目されてくるであろう。

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*Received on February 18, 2007, accepted on February 20, 2007*

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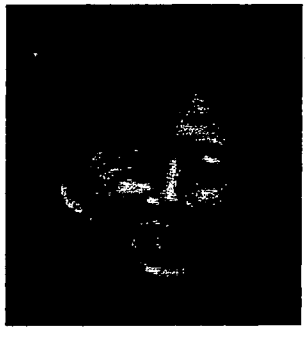

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BRAIN RESEARCH 1179 (2007) 1–15

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Research Report

# Distributions of glucuronyltransferases, GlcAT-P and GlcAT-S, and their target substrate, the HNK-1 carbohydrate epitope in the adult mouse brain with or without a targeted deletion of the GlcAT-P gene

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ARTICLE INFO

*Article history:*
Accepted 4 May 2007
Available online 16 May 2007

*Keywords:*
Glycosyltransferase
Glycosylation
Knock-out mice
Adult brain
In situ hybridization histochemistry
Immunohistochemistry

ABSTRACT

The HNK-1 carbohydrate epitope, a sulfated glucuronic acid at the non-reducing terminus of glycans, is expressed on glycoproteins and glycolipids and modulates neurite outgrowth and synaptic plasticity by affecting the adhesive and anti-adhesive properties. It is known that the HNK-1 carbohydrate is synthesized through two key enzymes, glucuronyltransferases (GlcAT-P and GlcAT-S). In the present study, we investigated the localization of GlcAT transcripts and HNK-1 carbohydrate in the adult mouse brain with or without GlcAT-P gene using *in situ* hybridization histochemistry and immunohistochemistry. Region-specific expression patterns of both GlcAT transcripts were observed. Strong expression of GlcAT-P and moderate expression of GlcAT-S were seen in neuronal cells of several nuclei of limbic-related regions and of the sensory system and the cerebellum. It was shown histologically that the localization of HNK-1 carbohydrate paralleled the pattern of expression of GlcAT

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*Abbreviations:* GlcA, glucuronic acid; GlcAT-P, glucuronyltransferases P (AB055781); GlcAT-S, glucuronyltransferase S (AB055902); HNK-1, $HSO_3$3GlcAβ1-3Galβ1-4GlcNAc; NCAM, neural cell adhesion molecule; CNS, central nervous system; 3n, oculomoter nu.; aca, anterior commissure; AD, anterodorsal thalamic nu.; Arc, arcuate hypothalamic nu.; AO, anterior olfactory nu.; BST, bed nu. of stria terminallis; CA1–3, subfield CA1–3 of Ammon's horn; Cbn, cerebellar nu.; cc, corpus callosum; Cg, cingulate cortex; Cu, cuneate nu.; DB, diagonal band; DC, dorsal cochlear nu.; DG, dentate gyrus.; DM, dorsomedial hypothalamic nu.; Ect, ectorhinal cortex; En, endopiriform cortex; fr, fasciculus retroflexus; Gl, glomerular layer of olfactory bulb; glc, granular cell layer of the dentate gyrus; Gr, gracile nu.; Hip, hippocampus; IC, inferior colliculus; IO, inferior olive; IP, interpeduncular nu.; LL, lateral lemniscus; LPB, lateral parabrachial nu.; LRt, lateral reticular nu.; LS, lateral septal nu.; MD, mediodorsal thalamic nu.; MG, medial geniculate nu.; MHb, medial habenular nu.; Mi, mitral cell layer of olfactory bulb; ml, molecular layer of the dentate gyrus; MPO, medial preoptic nu.; O, orbital cortex; PAG, periaquiductal gray; PB, parabrachial nu.; PBG, parabigeminal nu.; pcl, pyramidal cell layer of the hippocampus; Pir, piriform cortex; Pn, pontine nuclei; PoDG, polymorphic layer of the dentate gyrus; Pr5, principal sensory trigeminal nu.; PVA, anterior part of paraventricular thalamic nu.; Rt, reticular nucleus of the thalamus; RtTg, reticulotegmental nu. of pons; S, subiculum; SC, superior colliculus; SFO, subfornical organ; s-l, stratum lucidum; sm, stria medullaris of thalamus; s-o, stratum oriens; SO, superior olivary nu.; Sol, solitary tract nu.; Sp5, spinal trigeminal nu.; s-r, stratum radiatum; Tha, thalamus; VC, ventral cochlear nu.; VMH, ventromedial hypothalamic nu

0006-8993/$ – see front matter 
doi:10.1016/j.brainres.2007.05.012

transcripts in the brain. Additionally, the localization of HNK-1 carbohydrate was restricted partially in the brain of GlcAT-P-deficient mice, while the HNK-1 carbohydrate was widely distributed over most of the brain of wild-type mice. The present study provides a new framework for understanding the network constructed by the HNK-1 carbohydrate in the central nervous system.



## 1. Introduction

The central nervous system performs the most complex and dynamic biological functions. These functions are produced by complex neural connections resulting from the formation and maintenance of the vast array of synapses and cell–cell interactions, which depend on specific interactions between the extracellular matrix and cell membrane components. Carbohydrates on glycoproteins and glycolipids play important roles in a number of these interactions by enhancing either the adhesive or anti-adhesive properties of molecules involved in cellular adhesion (Pizzorusso et al., 2002; Rhodes and Fawcett, 2004). The HNK-1 carbohydrate epitope, a sulfated trisaccharide, $HSO_3$3GlcAβ1-3Galβ1-4GlcNAc (Chou et al., 1986; Voshol et al., 1996) is found on a number of glycoproteins, including neural cell adhesion molecule (NCAM) (Ong et al., 2002), L1, myelin-associated glycoprotein (Kruse et al., 1984), tenascin-C, and tenascin-R (Kruse et al., 1985), tissue plasminogen activator (Zamze et al., 2001), and glycolipids (Chou et al., 1986). Cell-biologically, the HNK-1 carbohydrate is thought to function in the modulation of neurite outgrowth (Martini et al., 1992), adhesion between neurons and glial cells (Kunemund et al., 1988), and synaptic plasticity (Dityatev and Schachner, 2003; Yamamoto et al., 2002). On the other hand, there have been few investigations concerning the HNK-1 carbohydrate in the adult brain *in vivo* except for ones focused on the hippocampus. One report showed that the immunoreactivity of the anit-HNK-1 carbohydrate antibody is detected as diffuse staining in the neuropil and individual somata in almost the whole brain (Yamamoto et al., 1988), but there is little knowledge about critical sites where the HNK-1 carbohydrate is expressed and to which it is conveyed in the brain network. The present study revealed critical regions expressing the HNK-1 carbohydrate based on histological observations and revealed the distributions of mRNAs of enzymes catalyzing HNK-1 synthesis. Two glucuronyltransferases, GlcAT-P and GlcAT-S, are key enzymes in the biosynthesis of HNK-1 carbohydrate, and catalyze the transfer of glucuronic acid (GlcA) from UDP-GlcA to Galβ1-4GlcNAc (Seiki et al., 1999; Shimoda et al., 1999; Terayama et al., 1997, 1998). Recently, we characterized the acceptor specificities of the two glucuronyltransferases using various oligosaccharides, suggesting the possibility that the two glucuronyltransferases synthesize structurally different HNK-1 carbohydrates (Kakuda et al., 2005). While it has been reported that GlcAT-P catalyzes HNK-1 synthesis mainly in the brain (Terayama et al., 1998), there have been no investigations of the specific distributions of each GlcAT transcript, and it has not been possible to discriminate between the HNK-1 carbohydrate produced by GlcAT-P and that produced by GlcAT-S. Recently, we generated GlcAT-P-deficient mice (Yamamoto et al., 2002), which permit us to determine the region-specificities of HNK-1 carbohydrate based on the expression of either GlcAT transcript in the brain. In the present study, we investigated the formation of the HNK-1 carbohydrate network in the central nervous system (CNS).

## 2. Results

### 2.1. *Distribution of GlcAT transcripts and HNK-1 in the adult mouse brain*

In normal adult mouse brain, most of the cells labeled with GlcAT-P and GlcAT-S cRNAs were ones showing representative neuronal shapes among cells stained with thionine. Table 1 shows scores based on a comparison of the relative signal intensities not only among different brain regions but also between GlcAT-P and GlcAT-S riboprobes, and Fig. 1 shows representative sections. There was no discrepancy concerning the levels of the transcripts between the present *in situ* hybridization and the previous Northern blot analyses (Terayama et al., 1997; Yamamoto et al., 2002). GlcAT-P mRNA was expressed widely in the mouse brain, while the expression of GlcAT-S mRNA was restricted. Regarding the distribution of GlcAT-P mRNA, especially, very strong labeling of neurons was detected in several nuclei of limbic-related regions, several sensory systems, and the cerebellum. Among the limbic-related regions, the anterior olfactory nucleus (AO; Fig. 1A-b), the piriform cortex (Pir; Fig. 1A-c), the lateral septum (LS; Figs. 1A-c and i), the hippocampus (Fig. 1A-d), the habenular nucleus (MHb; Fig. 1A-d), and the interpeduncular nucleus (IP; Fig. 1A-e) expressed GlcAT-P mRNA strongly. Among the sensory systems, there were the intense signals in the cochlear nucleus (VC and DC; Figs. 1A-f and g), the lateral lemuniscus (LL; Fig. 1A-j), and the inferior colliculus (IC; Fig. 1A-f) in the auditory system, the parabigeminal nucleus (PBG; Fig. 1A-j) in the visual system, the parabrachial nuclei (LPB; Fig. 1A-f) that function as gustatory relays, and the trigeminal sensory system (Pr5 and Sp5; Figs. 1A-f, g, and h). Concerning the expression of GlcAT-S mRNA, the transcript was detected in restricted areas among regions showing the expression of GlcAT-P mRNA and especially, the CA2/CA3-subfields (CA3; Fig. 1B-o), the ectorhinal cortex (Ect; Fig. 1B-o), and several nuclei of the thalamus (MD; Fig. 1B-n). In addition, the subfornical organ (SFO; Fig. 1B-n), the arcuate hypothalamic nucleus (Arc; Fig. 1B-o), and the medial geniculate of the thalamus (MG; Fig. 1B-p) expressed GlcAT-S mRNA very strongly, but expressed little GlcAT-P mRNA. We also investigated the distributions of GlcAT-P and GlcAT-S transcripts in the GlcAT-P-deficient mouse brain. There was, as expected, no signal with GlcAT-P cRNA probe in the GlcAT-P-deficient mouse brain. There was no difference in regional distributions