Review

Unraveling the mysteries of dendritic spine dynamics: Five key principles shaping memory and cognition

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Abstract: Recent research extends our understanding of brain processes beyond just action potentials and chemical transmissions within neural circuits, emphasizing the mechanical forces generated by excitatory synapses on dendritic spines to modulate presynaptic function. From *in vivo* and *in vitro* studies, we outline five central principles of synaptic mechanics in brain function: **P1: Stability** – Underpinning the integral relationship between the structure and function of the spine synapses. **P2: Extrinsic dynamics** – Highlighting synapse-selective structural plasticity which plays a crucial role in Hebbian associative learning, distinct from pathway-selective long-term potentiation (LTP) and depression (LTD). **P3: Neuromodulation** – Analyzing the role of G-protein-coupled receptors, particularly dopamine receptors, in time-sensitive modulation of associative learning frameworks such as Pavlovian classical conditioning and Thorndike's reinforcement learning (RL). **P4: Instability** – Addressing the intrinsic dynamics crucial to memory management during continual learning, spotlighting their role in "spine dysgenesis" associated with mental disorders. **P5: Mechanics** – Exploring how synaptic mechanics influence both sides of synapses to establish structural traces of short- and long-term memory, thereby aiding the integration of mental functions. We also delve into the historical background and foresee impending challenges.

Keywords: dendritic-spine, structural plasticity, associative learning, working memory, schizophrenia, autism spectrum disorder (ASD)

1. Introduction

Synapses are specialized cellular junctions in the brain where neurons communicate with one another primarily through chemical transmission. Spine synapses, which use glutamate as a transmitter and form on dendritic spines, are the most prevalent type of chemical synapse in the brain, making up 90% of excitatory synapses and 5% of brain volume. They are found throughout the brain and are most dense in the hippocampus, cortices, and striatum.[1)] The spine is the most actin-rich structure in the brain, and contains similar levels of actin to smooth muscle cells.[2)] The shape, actin content, and motility of these

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Non-standard abbreviation list: AC1: adenylate cyclase 1; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASD: Autism spectrum disorder; BDNF: brain-derived neurotrophic factor; CaMKII: calcium-calmodulin-dependent protein kinase II; cAMP: cyclic adenosine monophosphate; CS: conditioned stimuli; EPSP: excitatory postsynaptic potential; ERK: extracellular signal-regulated kinase; FRET: Förster resonance transfer; Gi,o: inhibitory G-protein; GPCR: G-protein-coupled receptor; Gs: G-proteins; LTD: long-term depression; LTE: long-term spine enlargement; LTM: long-term memory; LTP: long-term potentiation; NAc: nucleus accumbens; NMDA: N-methyl-D-aspartate; PKA: protein kinase A; PKC: protein kinase C; PREST: pressure sensation and transduction; PSD: postsynaptic density; RPE: reward prediction error; RL: reinforcement learning; SNARE: soluble N-maleimide sensitive fusion attachment *protein* receptor; STDP: spike timing-dependent protocol; STE: short-term spine enlargement; STM: short-term memory; STP: short-term potentiation; STSP: short term synaptic plasticity; TTX: tetrodotoxin; VTA: ventral tegmental area.

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synapses are evolutionarily conserved across species.[3)–5)] Humans possess 100 trillion spine synapses.[6)] Spine synapses express hundreds of key molecules essential for cognitive functions such as perceptual and behavioral decision-making, attention, emotion, language, reasoning, and planning. Notably, although a similarly large number of digital parameters are utilized in artificial intelligence, it is questionable whether they can produce the highly adaptive general intelligence and emotional experiences observed in the human brain.

Cognitive function is often associated with the development of neural activity patterns through learning, leading to the formation of internal representations of information.[7)] These transitions between brain states are shaped by a variety of neural activities, including neuronal firing, synaptic dynamics, intrinsic membrane properties, excitatory-inhibitory balance, and glial cell activity, all of which contribute to this process.[8)–13)] In particular, spine synaptic dynamics are vital for the formation of cell assemblies associated with individual synapses during associative learning.[14)–16)] Such assemblies can rapidly propagate throughout the brain via long axons that end with synapses.[17),18)] Moreover, numerous cognitive processes are integrated across the entire nervous system.[19)–21)] Investigating the origin of this self-unification, which may be facilitated by spine dynamics, is a crucial topic of inquiry in neuroscience.

Two-photon excitation microscopy is a valuable tool for examining the properties of spine synapses. It enables the imaging of brain tissues without toxicity because of the elimination of out-of-focal excitation, which is achieved with the help of femtosecond mode-locked lasers.[22),23)] Two major approaches have been used to examine spine synapses. The first approach involves the visualization of spines *in vivo* through time-lapse imaging via a cranial window, which can provide insights into spine dynamics in the living brain over a period of 1 day to 2 years.[24),25)] However, the time interval is long (1 day to 4 weeks), the activity histories and neuromodulatory tones are largely unknown, and mechanistic insights are correlational. The second approach involves the use of two-photon uncaging of caged-glutamate compounds, which is a single-synaptic method for examining the properties of single glutamatergic synapses and drawing causal relationships (Section 4.2).[26)] *In vivo* studies have mostly focused on the neocortex, which is located on the brain surface, whereas most *in vitro* studies use the hippocampus as a model system because it shows more pronounced plasticity. These two approaches are complementary, and the data should be carefully compared to elucidate the roles of spine synapses.[27)]

Since the publication of the 15 rules in 2010,[28)] there has been a plethora of new findings related to spine synapses, which have allowed us to distill the five core principles presented in Section 2 of this review. These principles offer essential insights into cognitive functionality and related dysfunctions, which are summarized in Section 3. Collectively, these are referred to as synaptic mechanics, underscoring the recognition that spine enlargement is fundamentally a mechanical process, and that structural plasticity or mechanics may underpin neural integrity. In Sections 4–8, we offer an in-depth exploration of each principle, delving into their historical context and potential pitfalls, which previously remained unaddressed. The subsections entitled "Unveiling our discovery (or development)" (Sections 4.2, 5.1, 5.4.2, 6.2, 6.3.2, 7.1, and 8.1) provide genuine insight into the backgrounds behind the discovery of each principle. Each section and subsection can be read independently, making this review accessible to both experts and non-experts in neuroscience and psychology, as well as researchers in artificial intelligence who are seeking a profound understanding of the underlying principles and engaging discussions on synaptic physiology in relation to cognitive function.

2. Five principles of synapse mechanics (Summary)

2.1. Structure-function relationship (P1: Stability – Section 4). The spine function is closely related to its structural attributes as shown in Fig. 1. Within the synaptic structure, AMPA and NMDA receptors play key roles in mediating rapid transmission and Ca^{2+} influx, respectively. The size of spine-head, ranging from 0.01–1 μm^3, emerges as a major determinant of synaptic connectivity and is linearly correlated with the functional expression of AMPA receptors (Fig. 1C–D). Similarly, the expression of NMDA receptors is also correlated, with the exception of significant expression in small spines exhibiting minimal AMPA receptor expression, known as "silent synapses" (Fig. 1E). Silent synapses possess the potential to form new functional connections through spine enlargement. These essential structure-function relationships were first discovered by two-photon uncaging (Section 4.1–4.3) but are now supported by numerous molecular bases (Section 4.4). Spine necks, which have a length ranging from

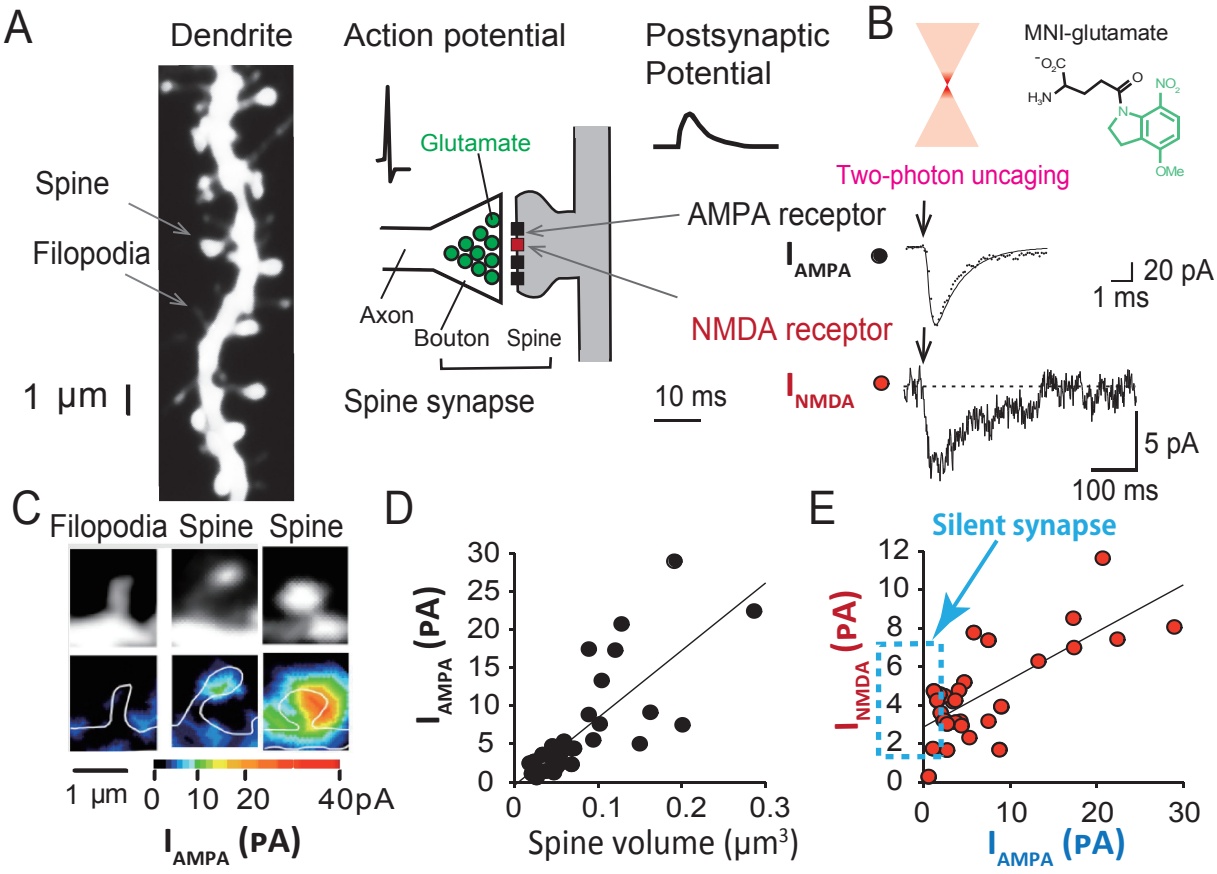

Fig. 1. Spine structure and function relationships (P1: Stability – Section 4). (A) Dendritic synaptic terminology. (B) MNI-glutamate and time-course currents mediated by the AMPA receptor (I_{AMPA}), which are 100 times faster than those mediated by NMDA receptors (I_{NMDA}). (C) Two-photon imaging and uncaging-induced currents used to map spine head sizes (upper panel) and glutamate sensitivity (lower panel), respectively, from whole-cell-recorded cells. Pseudo-color-coded peak current amplitudes are shown. (D) Linear structure-function relationship between I_{AMPA} and spine volume. (E) Peak amplitudes of I_{NMDA} in the same spines of several dendrites. Small spines show little I_{AMPA} but significant I_{NMDA}, consistent with the concept of "silent synapses". Adapted from Matsuzaki *et al.* (2001)[26] and Noguchi *et al.* (2005).[112]

0–3 μm and diameters between 0.05–0.9 μm,[1),29),30)] also influence the sensitivity of excitatory postsynaptic potentials (EPSPs),[31)] and constrain spine enlargement within spines. The slope of the structure-function relationship is adjusted by homeostatic scaling to stabilize spikes and NMDA receptor activity via transcriptional control (Section 4.5).[32),33)] Thus, the structure-function relationship serves as the foundation for understanding spine mechanics.

2.2. Activity-dependent structural plasticity (P2: Extrinsic dynamics – Section 5). Building upon the stable structure-function relationship described in P1, spine enlargement plays a crucial mechanical role in rendering spines efficient memory elements in the brain (Fig. 2). The combination of glutamate stimulation and postsynaptic depolarization triggers efficient Ca^{2+} influx through NMDA receptors,[34),35)] forming the basis of Hebbian associative learning, as described in Section 4.1. This process leads to spine enlargement and accumulation of AMPA receptors and supports synapse-specific associative learning (Section 5.1). These phenomena are observed as long-term potentiation (LTP) when many presynaptic fibers are collectively stimulated, and EPSPs are measured. Such stimulation can be repetitive high-frequency stimulation of presynaptic fibers (tetanus), or a spike timing-dependent protocol (STDP), in which pre- and post-synaptic neurons synchronously fire.[36)] This synapse-selective Ca^{2+}

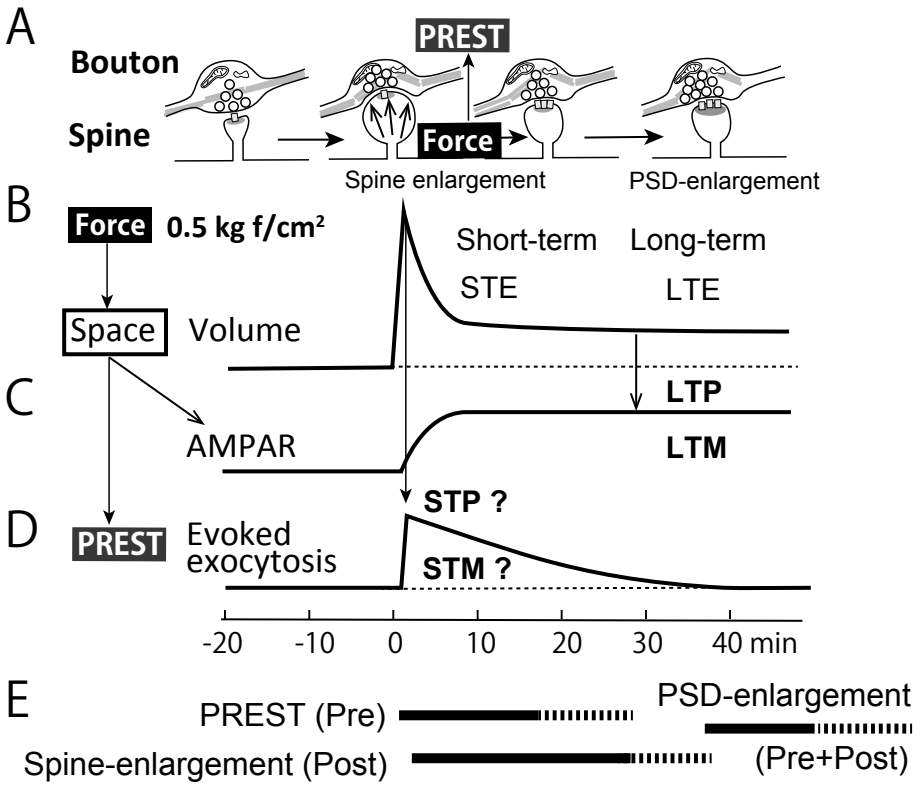

Fig. 2. (Color online) Activity-dependent structural plasticity (P2: Extrinsic dynamics – Section 5). (A) Force of spine enlargement and its effects on the presynaptic boutons. (B) Short- and long-term enlargement caused by force. (C) Gradual accumulation of AMPA following onset of long-term enlargement. (D) Presynaptic pressure sensation and transduction (PREST) effects induced immediately by the force of spine enlargement. PREST effects can last 10–30 minutes, and results in short-term potetiation (STP) and short-term memory (STM). (E) Three mechanisms of functional expression: PREST, which is presynaptic; Spine enlargement, which is postsynaptic; and PSD enlargement, which accompanies enlargement of the active zone (AZ).

influx activates calcium-calmodulin-dependent protein kinase (CaMKII) and reorganizes actin scaffolds, generating a muscle-like force for enlargement (Fig. 2).[37] The stabilization of the early phase of enlargement (STE) results in the late phase of enlargement (LTE) through structural and mechanical processes (Section 5.2). As a result, LTE is regulated by various chemical factors, including protein kinase A (PKA), protein kinase C (PKC), brain-derived neurotrophic factor (BDNF), and protein synthesis for longer persistence. The extent of enlargement varies among spines, with smaller spines showing more pronounced effects, which may reflect a write-protection mechanism cascade model. Similar spine plasticity has been found in the hippocampus, neocortex, and striatum (Section 5.3). Synaptic activity also causes spine shrinkage and long-term depression (LTD) through mechanisms that are in many ways asymmetric with enlargement (Section 5.4). Both spine enlargement and shrinkage of a single spine generate spreading factors along the dendrites, supporting clustered, synergistic, and competitive synaptic dynamics (Section 5.5), representing non-Hebbian plasticity.

2.3. Time-dependent modulation of extrinsic dynamics by G-protein-coupled receptors (P3: Neuromodulation – Section 6). In Pavlovian and Thorndike conditioning, as defined in Section 6.1, spine enlargement is regulated by G-protein-coupled receptor (GPCR) agonists within a specific time frame, following conditioned stimuli (CS) and operant behaviors (Fig. 3). Activation of adenylate cyclase 1 (AC1) by GPCR agonists is delayed until the delivery of a reward or punishment, triggering the production of cytosolic cyclic adenosine monophosphate (cAMP). With repeated training, the accumulation of cAMP stimulates PKA, promoting spine enlargement. This window for efficient AC1

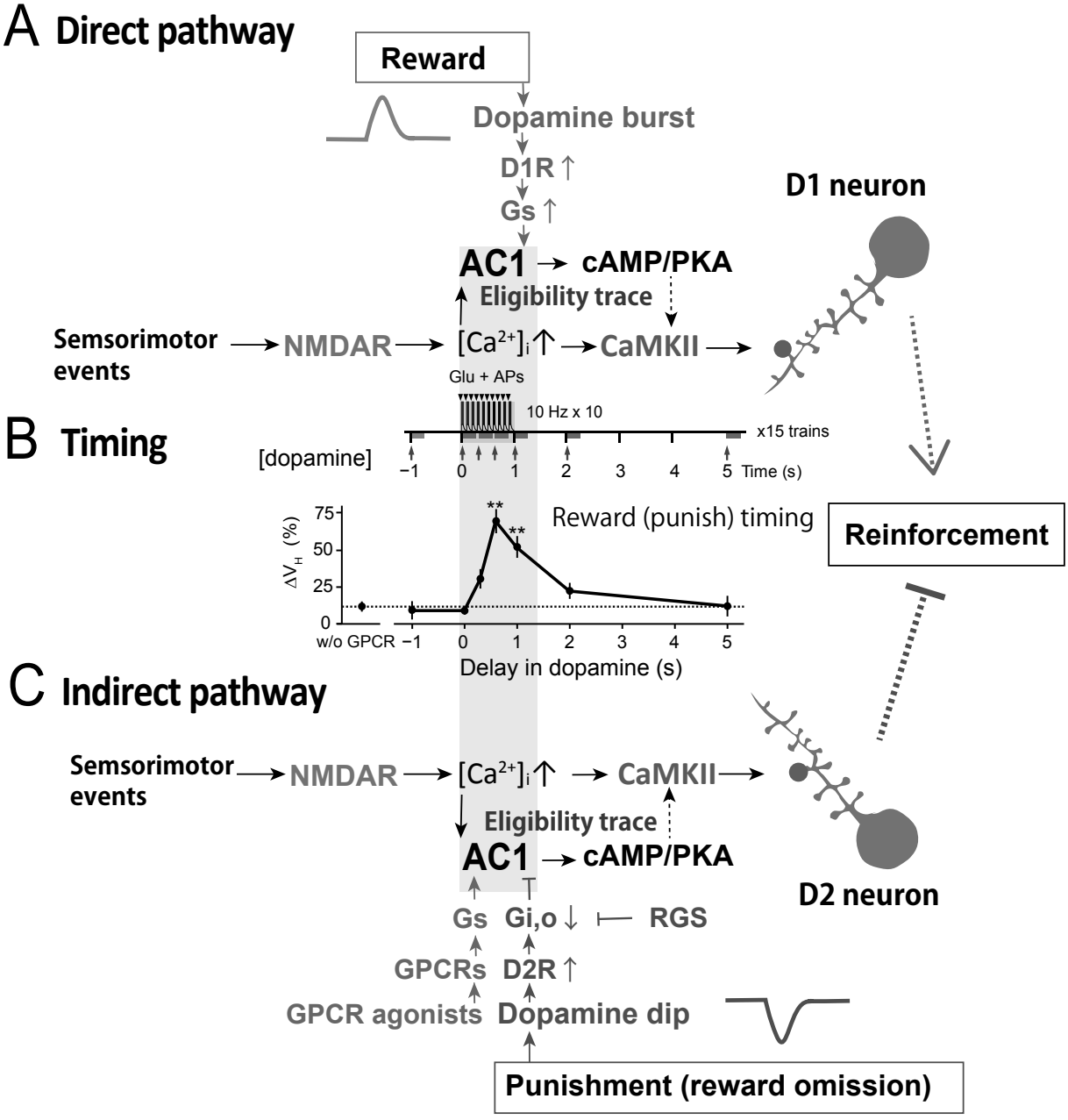

Fig. 3. (Color online) Time-dependent modulation of extrinsic dynamics by GPCRs (P3: Neuromodulation – Section 6). (A) Sensorimotor stimuli (CS) for conditioning and their effects on $[Ca^{2+}]_i$ levels and subsequent Gs activation in D1 neurons in the direct pathway. (B) Molecular mechanisms underlying the eligibility trace for conditioning, including Ca^{2+}-dependent AC1 and its ability to detect temporal contiguity between $[Ca^{2+}]_i$ and GPCR signals for spine enlargement. (C) The dichotomous nature of RL in the indirect pathway, which involves both AC1 activation by $[Ca^{2+}]_i$ increase via STDP and Gs activation by disinhibition of Gi,o. This type of RL enables the evaluation of the CS in a manner that depends on both the memory and the activity of the two pathways. The additional molecular complexity of the D2 receptors (red) renders punishment learning vulnerable, and could be a potential pathophysiological basis for psychosis.

stimulation and conditioning is narrow, falling within the 0.5–2 second range, mirroring the reward timing in animals and the persistence of an eligibility trace in machine reinforcement learning (RL).[38] GPCRs can couple with either stimulatory heterotrimeric G-proteins (Gs) or inhibitory G-proteins (Gi,o), which

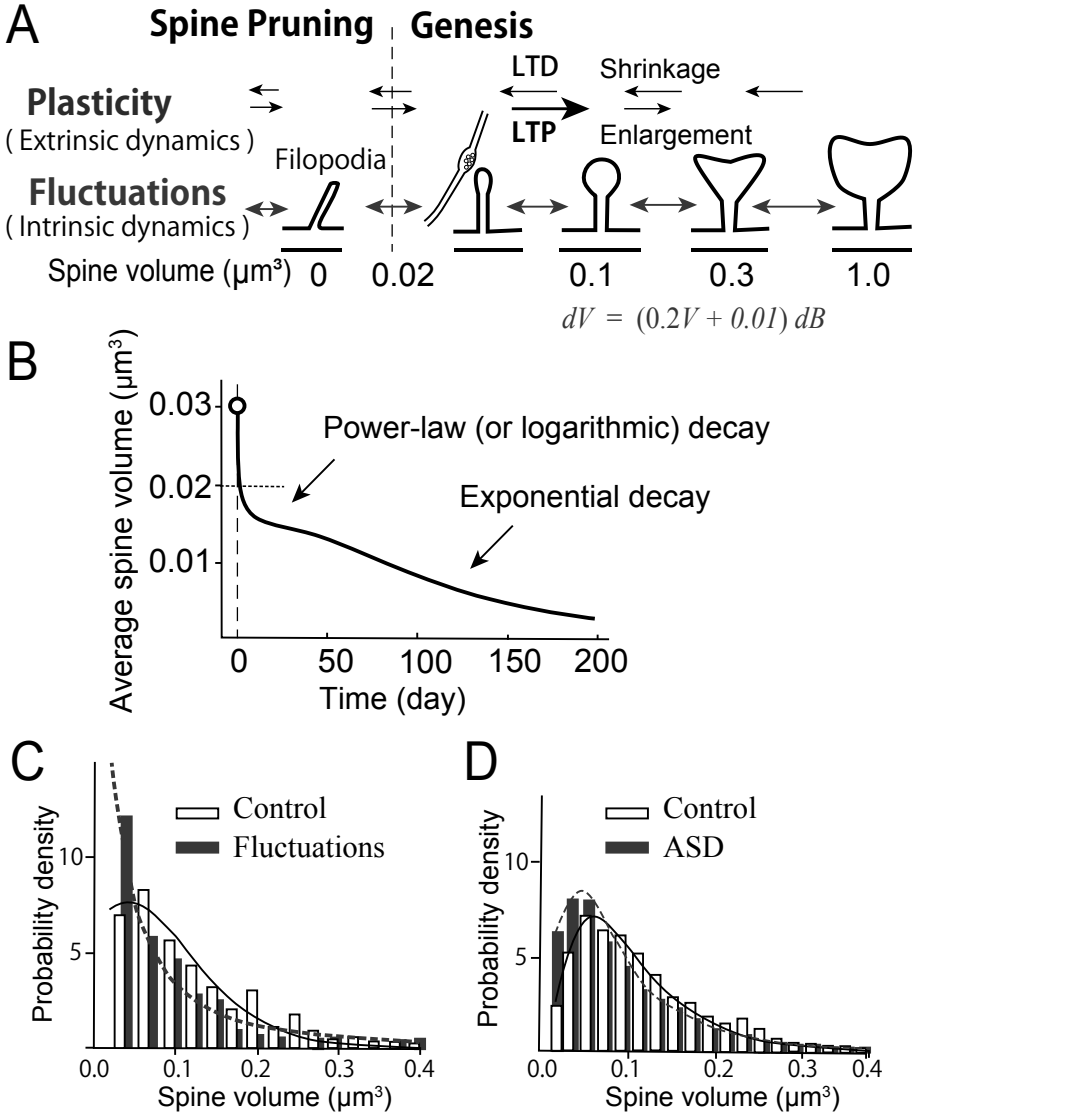

Fig. 4. (Color online) Intrinsic dynamics and memory management (P4: Instability – Section 7). (A) Intrinsic dynamics of spines represent their daily stability, which is more prevalent than extrinsic dynamics (plasticity) and leads to the normalization of weight distributions of many spines to unimodal, skewed, and heavy-tailed distributions (long-normal-like distributions) (C, D); many unused spines are pruned spontaneously, resulting in the formation of new spines and filopodia. (B) Time courses of the average volume of spines, with an initial volume of $0.03\,\mu m^3$, can be described by the stochastic differential equation: $dV_t = (0.2\,V_t + 0.01)\,dB_t$. Here, V_t represents the spine-volume process and B_t denotes Brownian motion. Initially, the average volume exhibited a slow decay following a logarithmic or power-law pattern for approximately 30 days. Subsequently, the decay transitions exponentially. When calculating the average volume, the spine volumes were considered to reach zero when crossing a boundary of $0.02\,\mu m^3$.[39] The average spine volumes (V_t) smaller than 0.02 approximately predict the persistence probability as $V_t/0.15$. (C) Distributions formed by the intrinsic dynamics of spines (blue, log-normal-like) in the presence of plasticity (control). (D) Small spine volumes in ASD models (blue) and wild-type mice (white). Adapted from Yasumatsu *et al.* (2008)[39] and Ishii *et al.* (2018).[74]

expedite spine enlargement by either increasing or decreasing agonist concentrations, respectively (Section 6.2–6.3). These circuits facilitate a dichotomous form of RL, allowing for the separate storage and retrieval of positive and negative memories, contingent on the neuromodulation status, which diverges from the approach taken in machine RL. This process may underpin the basis for emotion (Section 6.4).

2.4. Intrinsic dynamics and memory management (P4: Instability – Section 7). Spines, being

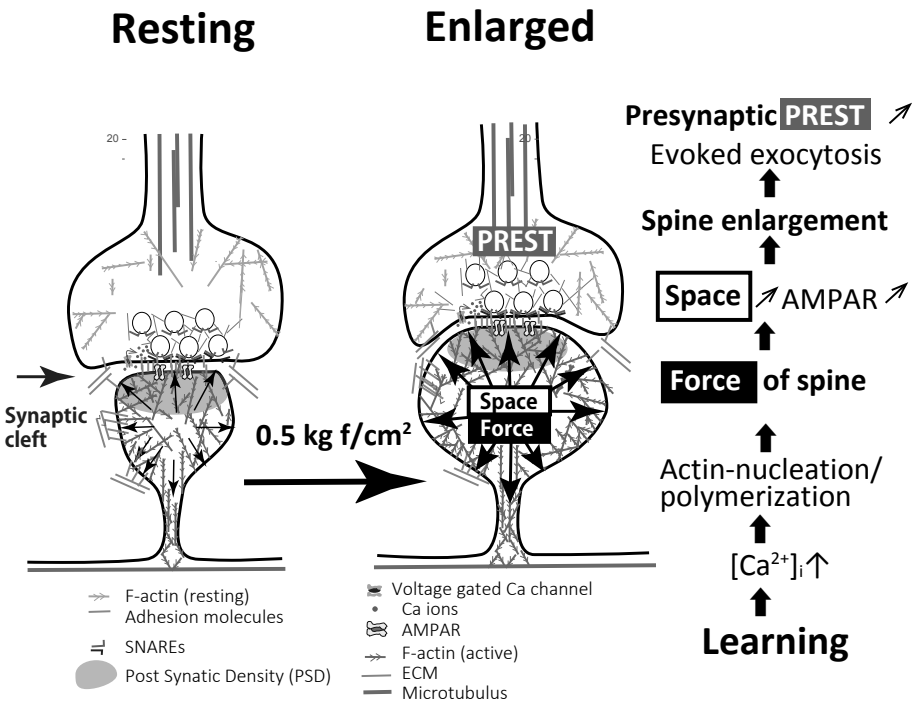

Fig. 5. (Color online) Mechanical transmission of spine synapses (P5: Mechanics – Section 8). The force generated by actin polymerization has two major effects: expansion of spines and pushing of presynaptic terminals. The force of pushing was measured to be 0.5 km f/cm^2, which is similar to muscle contraction. This force is fundamental to spine enlargement and pushing of presynaptic terminals. Pushing of the presynaptic terminal represents the backpropagation of associative memory information, although specific actions are not fully understood. Adapted from a modified version of Kasai *et al.* (2023).[53]

small living structures, exhibit slow intrinsic dynamics or inevitable fluctuations when we consider continual learning over days (Fig. 4).[39)–46)] These intrinsic dynamics provide an accurate measure of the long-term stability of spine enlargement and LTP, independent of synaptic activity, thus setting them apart from extrinsic synaptic dynamics (P2 and P3). They normalize synaptic weight distributions that are skewed towards smaller spines and serve as a homeostatic mechanism for connectivity weight distributions (Section 7.2). These processes also encourage new memory formation by facilitating spine generation (genesis) and elimination (pruning), with rates varying by age and method.[24),25),47)] Intrinsic dynamics may lead to the gradual degradation of memory over time, which is countered by memory reactivation processes within recurrent networks (Section 7.3). Characterized by synaptic instability, intrinsic dynamics can result in the development of small spines or "spine dysgenesis", especially noticeable in conditions like autism spectrum disorders (ASDs).[48)] Spine genesis commences with the formation of motile filopodia, typically measuring 0.5–3 µm in length and 0.5 µm in diameter, which are often devoid of synaptic contact.[47),49)–52)] While spine genesis primarily depends on intrinsic dynamics, it is also influenced by extrinsic dynamics and can be modulated by neurosteroids (Section 7.5).

2.5. Mechanical transmission of spine synapses (P5: Mechanics – Section 8). Spine enlargement exerts an expansive force, driving neurotransmitter release from the presynaptic terminal for a duration of 20–30 minutes (Figs. 2 and 5).[37)] This process, termed "pressure sensation and transduction" (PREST), is a back propagation of learning effect, and only beginning to be fully understood. PREST mechanisms are utilized to detect or monitor spine tip expansion (STE) and may contribute to short-term potentiation (STP) and other closely related phenomena (Section 8.2).[53)] Evidence suggests a role of STP in synaptic working memory, where synaptic mechanisms aid in memory maintenance and associativity. Traditional short-term synaptic plasticity (STSP), such as facilitation and depression, may operate under similar mechanisms.[8)]

Unlike STSP, working memory involves the association of new incoming information with long-term memory (LTM) already stored, which often transforms into LTM (Section 8.3). The involvement of STP in working memory suggests that spine mechanics contribute to the process of self-unification (Section 8.4).

3. Cognitive function and dysfunction (Summary)

3.1. Synaptic underpinnings of mental function. The maintenance of cognitive stability relies on the structure-function relationship (Fig. 1) (P1: Stability − Section 4). Spine enlargement facilitates fast synaptic transmission mediated by AMPA receptors, whose expression levels are proportional to spine volumes. Through individual spine enlargement, spines undergo independent potentiation, leading to enhancements in both structure and function for the formation of cell assemblies (Fig. 2) (P2: Extrinsic dynamics − Section 5). As the major excitatory synapses in all brain regions, spines are involved in both explicit (declarative) and implicit (nondeclarative) memories. The degree of spine enlargement varies from one spine to another, providing write-protection mechanisms, as illustrated in the synapse cascade model (Section 5.3.5). Neuromodulators, particularly those applied shortly after stimuli for synaptic associative learning and at reward or punishment timing, accelerate spine enlargement (Fig. 3) (P3: Neuromodulation − Section 6), resulting in conditioned and emotional learning.[54),55)] Neural circuits formed by spine synapses undergo dichotomous RL throughout development, which becomes the basis of emotion (Section 6.4.1).

Small spines or filopodia are mostly generated by intrinsic dynamics (P4: Instability) and readily enlarged by learning stimuli (P2: Extrinsic dynamics), facilitating the formation of new functional connections and memory from silent synapses.[56)] The abundance of new memories is reflected in the predominance of small spines due to fluctuations (Fig. 4) (P4: Instability − Section 7). Intrinsic dynamics are slow and explain the persistence of memory for several days (Section 7.2). However, spines and memory can persist for weeks or years after reactivation (Section 7.3).[24)] Spine enlargement and the resulting mechanical transmission may lead to rapid associative plasticity (Fig. 5) (P5: Mechanics − Section 8), which can form short-term working memory and bind various representations of cognitive function, with long axonal projections connecting the entire distance of the brain. As with short-term enlargement, short-term memory (STM) often extends to LTM through reactivation in healthy individuals.[57)]

3.2. Synaptic underpinnings of mental disorders. The structure-function relationship (Fig. 1) (P1: Stability − Section 4) is robustly supported, even for disorders. Subtle abnormalities in spine shape and distribution can lead to serious mental disorders, and deviations in spine structure and mechanics may serve as a common underlying factor for such disorders.

Schizophrenia is characterized by both positive and negative symptoms as well as cognitive dysfunction. While positive symptoms (psychosis), such as delusions and hallucinations, are used to diagnose schizophrenia, cognitive dysfunction is centered on deficits in executive function, working memory, and attention. Genetic links have been found between schizophrenia and synaptic plasticity genes, suggesting that spine enlargement (P2) and mechanics (Fig. 5) (P5 Mechanics − Section 8) are major impairments.[58)] Spine numbers are often reduced,[59)–62)] particularly in small spines,[63)–66)] and the number of synaptic vesicles is also reduced in presynaptic terminals in schizophrenia.[67),68)] Psychosis in schizophrenia is caused by an impairment in punishment learning that removes improper perceptions and thoughts (Section 6.3.4), because D2-mediated learning is vulnerable (P3: Neuromodulation − Section 6). The signaling cascade of punishment learning is intricate and vulnerable to stress (Fig. 3), but it can be restored by antipsychotics such as dopamine D2 receptor (D2R) antagonists.[69),70)]

ASD is diagnosed based on early onset communication and language disabilities, repetitive behaviors, and behavioral inflexibility.[71)] Genetic linkages are often observed in synaptic scaffolders, adhesions, protein synthesis, and lipid metabolism. Common synaptic symptoms include small spine sizes, spine dysgenesis, an increase in spine numbers,[48),56),72)–74)] and spine turnover (P4: Instability).[75)–78)] Proteins involved in constitutive synaptic maintenance, such as neuroligin, FMR, Shank, and Neuroligin1, are often the cause of ASD.[71)] Therefore, exaggerated synaptic instability (P4) and the resulting abundance of small spines are considered to be one of the common pathogenetic mechanisms of ASD (Fig. 4) (Section 7.4).

Monoamine supplementation is a typical treatment for mood disorders.[79)] However, its gradual amelioration of depressive symptoms suggests the

involvement of synaptic plasticity (P3) and the spine generation process (P4), both of which are regulated by neuromodulators (Section 7.5.3). In conditions such as dementia[80)–82)] and Huntington's disease,[83)] cognitive decline precedes neuronal cell death and involves various functional and structural abnormalities of the spine. Moreover, spine loss is common under these conditions.

4. Spine structure-function relationship (P1: Stability)

4.1. Examining the "Perils of LTP" and dendritic spines. Donald Hebb postulated synaptic associative plasticity in 1949. He suggested that when the axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change occurs in one or both cells, enhancing A's efficiency in firing B.[14)] Hebb further asserted that this type of synapses formation enables the development of specific neural activity patterns through the "fire together, wire together" principle, contributing to the establishment of internal representations.[7)]

In 1973 Bliss and Lomo reported that tetanic stimulation of a presynaptic fiber bundle using a metal electrode leads to LTP of EPSPs. This effect initiates within one minute and persists from one hour up to three days.[84),85)] From that time forward, the question of whether electrode-induced LTP embodies Hebbian plasticity has been of significant interest.[86)–88)] The validation of selective stimulation of the pathway was achieved using two stimulation electrodes positioned both upstream and downstream of the cells under recording (Fig. 6A).[87)] However, several concerns arose regarding the correlation between LTP and Hebbian synaptic plasticity. Firstly, the pathway-specific induction of LTP does not necessarily equate to synapse selectivity, as the two-pathways must be the immediate vicinity of each other for synaptic selectivity. Yet, it is probable that these two pathways are spacially separated (see Fig. 6A for a simplified version), considering that the two electrodes rarely stimulated overlapping set of fiber populations. In reality, electrode-induced LTP has been reported to diffuse to adjacent synapses up to a distance of 10 µm.[89)–91)] Secondly, electrodes also stimulate GABAergic (Section 5.4.3) and monoaminergic fibers (Section 6.2.1) (Fig. 6A), which further complicates the interpretation. Furthermore, neither pre- nor postsynaptic components are selectively stimulated and recorded to properly determine the locus of expression.

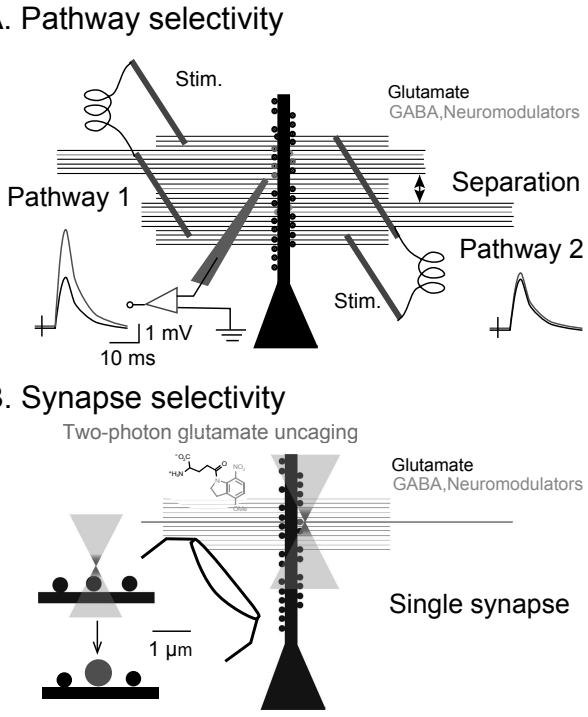

Fig. 6. (Color online) Pathway and synaptic selectivity of LTP/LTD and spine enlargement. Presynaptic fibers consist of approximately 80% glutamate (gray), 10% GABA (cyan), and 10% neuromodulators (cyan). (A) Two electrodes are utilized to stimulate one pathway upstream (Pathway 1) and the other downstream (Pathway 2). However, the two electrodes invariably stimulated distinct fiber populations, suggesting some degree of separation between the spines associated with the two pathways. The fiber organization depiction is simplified to illustrate the concept of "separation". In reality, the stimulated fibers are dispersed and the two pathways are intermingled. This separation complicates the examination of synaptic selectivity. In addition, the electrodes stimulate presynaptic fibers containing GABA and neuromodulators, preventing selective stimulation of glutamatergic input. (B) With the application of two-photon glutamate uncaging, selective stimulation of individual spines is achievable, enabling synapse-specific potentiation of glutamate sensitivity. This technique allows for precise control and investigation of individual synapse effects, contributing to a deeper understanding of the mechanisms driving synaptic plasticity.

Despite these limitations, the application of electrode-induced LTP has become an established and accessible methodology, facilitating the investigation of hundreds of chemical compounds and genetically altered mice.[92)] Over the past three decades, however, the field has faced significant challenges, referred to as the "perils" of LTP.[93),94)] Several crucial questions have emerged in this context: 1) Why does LTP involve a multitude of molecules? 2) Is LTP a singular phenomenon, as

suggested by the terminology? 3) Why do disagreements persist concerning the presynaptic or postsynaptic locus of expression? These questions are addressed in Sections 5.2.2–5.2.4.

Furthermore, a hypothesis concerning the role of dendritic spines in memory storage was proposed towards the end of the 19th century,[95] and the involvement of dendritic spines in LTP was similarly considered.[31),85),96),97] Nevertheless, definitive evidence supporting the role of spines remained elusive until 2001.

The aforementioned issues led to the development of two-photon uncaging of caged-glutamate compounds, which can release glutamate specifically at the tip of an individual, identifiable spine (Fig. 6B) (Section 4.2). This feature is beyond the reach of conventional one-photon excitation, which provokes a cone-like excitation and makes the selective stimulation of single spines unattainable in tissue preparations. Currently employed optogenetic methods also present constraints, as they are unable to stimulate a singular synapse owing to axonal branching, leading to the stimulation of synapses throughout the entire axonal path.

4.2. Unveiling our development of two-photon caged-glutamate compounds. In 1995, our laboratory (Kasai and Nemoto) embarked on the construction of a two-photon uncaging microscope for single-spine stimulation, leveraging our familiarity with the usage of one-photon caged compounds.[98)–100] During that period, all-solid-state mode-locked femtosecond lasers became accessible in the market. In 1997, Matsuzaki found that a commercially available caged-glutamate compound, γ-carboxy-nitrobenzyl (CNB), showed little two-photon excitation (2PE), and that a femtosecond laser (120 fs, 82 MHz, 720 nm, 4 mW for 0.6 ms) caused two-photon injury currents when they interacted with a dendrite.[101),102] To overcome this issue, we collaborated with Ellis-Davies to develop a two-photon-compatible caged-glutamate compound in 1997.[103] This endeavor presented a challenge, as the two-photon excitation (2PE) cross-section was only predicted by intuition at that time. Fortunately, in 1998, he synthesized γ-dimethoxy-CNB glutamate, which was marginally usable, motivating us to persist with the project. In 1999, the Roger Tsien lab reported two-photon glutamate uncaging using bromo-hydroxycoumarin (Bhc) glutamate, but the photolysis of glutamate was too slow, and glutamate uncaging had little spatial resolution.[104] Concurrently, the Corrie lab synthesized nitroindolino-glutamate (NI-glutamate) to improve light absorption in 2000.[105] Based on this work, the Ellis-Davies lab synthesized 4-methoxy-7-nitroindolinyl-glutamate (MNI-glutamate, Fig. 1B) and we found it effectively induced clean AMPA receptor-mediated currents with two-photon uncaging, as reported in 2001.[26] Independently, the Corrie lab synthesized MNI-glutamate, and reported its utility for one-photon uncaging.[106]

After optimizing our microscopic system to achieve diffraction-limited resolution for two-photon uncaging, Matsuzaki applied it to hippocampal CA1 pyramidal neurons and found that the expression of functional AMPA receptors was nearly proportional to the spine volume in slice culture preparations.[26] Three-dimensional mapping of uncaging-induced currents (Fig. 1C) was performed to confirm that glutamate sensitivity accumulated as a hotspot in the spine, and maximal currents, which reflect the number of glutamate receptors, showed a linear correlation with spine head size (Fig. 1). Although MNI-glutamate has a usable 2PE of 0.06 GM, it blocks GABA receptors at the concentrations (4–12 mM) necessary for stimulation of single spines.[107] To address this issue, Ellis-Davies synthesized an improved caged-glutamate compound, 4-carboxymethoxy-5,7-dinitroindolinylglutamate (CDNI-glutamate) in 2007,[108] which has a 2PE of 0.6. A concentration of 2 mM enabled glutamate uncaging without blocking GABA receptors and eliciting action potentials.[37),70),103),109)–111]

Two-photon uncaging has a focal volume that can be measured by a fast AMPAR-mediated current with lateral and axial diameters of 0.6 and 1.4 µm, using a lens with a numerical aperture of 0.9. The duration of two-photon uncaging is 0.6 ms,[26] prolongation beyond this durations triggers the expansion of NMDA receptors activation beyond 1.4 µm laterally, and significantly stimulates extrasynaptic NMDA receptors in the dendritic shaft.[112] When we perform uncaging at the spine, EPSCs mediated by AMPA and NMDA receptors are selectively induced at the spine and exhibit similar time courses as miniature EPSCs (mEPSCs).[26),112] It is important to note that uncaging with longer durations leads to greater spread of receptor activation from synaptic sites. The laser power is judiciously restricted (4–6 mW for at 120 fs pulse width) to ensure that the induced EPSCs from uncaging exhibit amplitudes akin to those of mEPSCs.

4.3. Structure-function relationships. The Harris lab established a correlation between spine

volume and postsynaptic density (PSD) size using an electron microscope (EM).[29] Moreover, the Somogyi and Ottersen labs found correlations between PSD areas and AMPA receptor immunoreactivity using EM.[113),114] The function of individual spines, however, was unclear until 2001.[96),97] We then discovered the structure-function relationship using two-photon glutamate uncaging.[26] These findings have been confirmed in the hippocampus[115)–118] in the striatum,[110] and in the neocortex *in vivo*.[119] The number of functional AMPA receptors was estimated to be approximately 100 per spine on average.[26]

The linear structure-function relationship has been validated using presynaptic electrical stimulation and the quantal analysis of synaptic currents together with serial section EM (ssEM) in neocortical slices.[120] In the Huganir lab, molecular markers for AMPA receptor expression have been developed using knock-in mice with superecliptic pHluorin-tagged GluA1 (SEP-GluA1), which also confirmed the structure-function relationship[121] in the visual cortex[122] and in the barrel cortex.[123] Biotinylated AP-GluA2 was utilized to label AMPA-receptors without a bulky extracellular tag (SEP) in the hippocampus.[124] The structure-function relationship suggests that the diversity of spine sizes along dendrites represents the strength of synaptic connections and that long-term changes in AMPA receptor expression are associated with spine structural alterations.

It is essential to highlight that NMDA receptors behave differently from AMPA receptors in terms of their association with spine volume. Despite their presence in small, thin spines, NMDA receptors display significant expression, whereas AMPA receptors are sparse.[112),114),125] This has been confirmed by Ca^{2+} imaging, as NMDA receptors' high affinity for glutamate compromises spatial resolution[112] due to the spillover effects.[126] This characteristic allows smaller spines to demonstrate larger increases in intracellular calcium concentration ($[Ca^{2+}]_i$), consistent with the concept of a "silent synapse" (Fig. 1E). Such synapses contain NMDA receptors, but lack AMPA receptors, making them preferential sites for LTP induction.[127),128] Silent synapses comprise small, thin spines with synaptic connections, whereas filopodia lack synaptic connections as well as AMPA and NMDA receptors.[112] Silent synapses are more common in younger animals.[129)–132] For spines to be selected during learning, even nascent spines should possess sufficient NMDA receptors for enlargement, while having minimal AMPA receptors to avoid interfering with network functions.[133]

Spine heads have particularly high concentrations of actin, similar to the smooth muscle cells found in brain micro-vessels.[2] At the tip of the spine head, spines form a connection with the presynaptic terminal via PSDs, where various scaffolding molecules initiate actin polymerization.[134] Actin filaments consist of individual actin subunits (G-actins) that can assemble or disassemble. At one end of the filament, referred to as the "plus end" or "barbed end", actin subunits are added to promote filament growth. At the other end, known as the "minus end" or "pointed end", the actin subunits are removed, resulting in filament shortening. This simultaneous process of actin subunit addition at the plus end and removal at the minus end creates a net movement, known as "treadmilling", of the actin filament. In spine heads, fibrous actins (F-actins) undergo treadmilling from the plasma membrane to the cytosol with a time constant of approximately 1 minute (Fig. 5). Elongation of F-actins is severed by cofilin in the cytosol,[135] contributing to the generation of protrusive forces and the formation of morphing and filopodia (spinule).[51),136),137] In addition, at the base of spine heads, a more stable actin pool with a slower turnover rate (20 minutes) exists, where F-actin binding proteins, such as α-actinin and myosin II, are more densely associated.[138),139]

Throughout the 20th century, the study of dendritic spines primarily focused on their necks.[31),140)–142] The defining features of spines are these fine and elongated necks, which possess a specialized actin organization.[143),144] These spine necks exhibit longer lengths in the neocortex than in the hippocampus and may amplify depolarization in the spine[30),120),145)–148] so much more than in the dendritic shaft.[1),149)–151] The narrow spine neck plays a crucial role in constraining diffusion between the spine head and dendritic shaft.[30),112] However, during spine enlargement (P2), spine neck thickening[30),112),152),153] and often twitching[37),109),152),154),155] can mitigate the enhancement of spine potentials under learning conditions while simultaneously increasing the availability of biochemical substances along the neck. As a result, spine necks are fine-tuned to be responsible for input before learning while becoming more efficient for structural plasticity during the learning process.

Finally, a structure-function relationship also exists in the presynaptic active zone (AZ), although the correlation is less pronounced than in the spine-

AMPA receptor relationship, and there is a substantial release probability (Pr), even with small synaptic contacts.[120),156)] This relationship serves as a foundation for postsynaptic silent synapses, such that glutamate is released from presynaptic terminals to postsynaptic NMDA receptors. Despite their weak correlations, boutons along the same axons and even synapses on the same bouton exhibit independence in terms of their structures and learning-related morphological plasticity.[157)]

4.4. Molecular basis of structure-function relationship. The structure-function relationship of AMPA receptors has been extensively investigated by several laboratories, including the Choquet and Nicoll labs, revealing a robust and elaborate molecular basis. Extrasynaptic AMPA receptors are abundant,[158)] and they exhibit lateral diffusion and binding to the PSD (diffusional trapping).[159)–164)] Certain auxiliary subunits of AMPA receptors,[165)] such as transmembrane AMPA receptor regulatory proteins (TARPs) and cysteine knot AMPA receptor-modulating proteins (shisa), possess a PDZ-binding motif that interacts with the PDZ domain of PSD proteins.[166)–169)] Such interactions may also be mediated by liquid-liquid phase separation.[170)]

In contrast to AMPA receptors, NMDA receptors can be tightly bound to PSD proteins via the C-terminal PDZ ligand domain.[171),172)] The PSD is composed of one to five nanomodules with diameters that are correlated with spine sizes, as revealed by STED microscopy.[173)–175)] Small spines with only one cluster contain NMDA receptors, but not AMPA receptors, indicating that the binding of NMDA receptors to the cluster is stronger.[176)] In clusters containing both AMPA and NMDA receptors, NMDA receptors are located at the center, surrounded by AMPA receptors.[177)]

The existence of AMPA-silent synapses requires consideration of the fact that AMPA receptor-mediated currents are not solely determined by the total number of receptors in the PSD but also by the receptor density in the area directly opposing the AZ release site because of their low glutamate affinity.[178),179)] Super-resolution microscopy has revealed the formation of trans-synaptic nanocolumns,[176),178),180)–182)] which may be facilitated by cell adhesion molecules and extracellular regions of AMPA receptors extending far into the synaptic cleft, participating in molecular interactions with extracellular components.[183),184)] This *trans*-synaptic clustering of AMPA receptors contributes to the density of receptors opposing the AZ release site.[168),185)] This concern does not apply to NMDA receptors, as they exhibit high affinity for glutamate.

4.5. Homeostatic scaling. Homeostatic scaling, discovered by the Turrigiano and Nelson labs, is a mechanism that adjusts the strength of all excitatory synapses of a neuron up or down based on activity levels to stabilize firing.[32),33),186)] This process is induced by the chronic blockade of spikes using tetrodotoxin (TTX) and involves a Ca^{2+}-dependent global increase in postsynaptic receptor abundance. Glial TNFα secretion appears to play a role in promoting AMPA receptor expression over the course of a few days without affecting LTP and LTD.[187)] Homeostatic scaling relies on transcriptional and translational control and typically occurs with a delay of approximately one day.[188)] During homeostatic scaling, spine structures do not necessarily change; instead, the slope of the structure-function relationship is regulated by activity levels.[189),190)] In fact, no global scaling in spine volume is observed following the complete blockade of the spike by TTX over a week.[39),43)] Interestingly, homeostatic scaling of spine sizes can also be induced as a response of spine fluctuations (P4: Intrinsic dynamics) to activity-dependent plasticity (P2: Extrinsic dynamics).[191)]

While homeostatic scaling is a well-defined phenomenon in an entire neuron, it is an example of a broader concept known as homeostatic plasticity.[189),190)] Branch-specific homeostatic plasticity of spine size down-scaling may be induced by optogenetic stimulation for one day in a transcription-dependent manner,[192)] or up-scaling may occur due to sensory deprivation via TNFα.[193),194)] Homeostatic plasticity is also proposed for presynaptic terminals.[195)] These plasticity processes may serve as a local normalization mechanism if the subcompartments of cells act as a unit.[196)]

5. Activity-dependent structural plasticity (P2: Extrinsic dynamics)

5.1. Unveiling our discovery of spine enlargement. We discovered rapid enlargement of stimulated spines using two-photon uncaging, which was reported in 2004 as described in the next paragraph.[128)] Prior to this work, gradual and dispersed alterations in spine structure had been reported. EM studies comparing populations of preparations with and without tetanic electrical stimuli that induced LTP after 0.5–2 hours reported increases in spine surface areas in the dentate gylus[97),197)] (but not in a more quantitative study in

the CA1region[198]), as well as poly-ribosomes,[199] and spine branching.[200] Two-photon microscopy revealed that such stimuli led to generation of filopodia or spines with a notable 10 minute delay.[201),202] While these findings were noteworthy, the fundamental question remained: whether the structurally altered spines were directly stimulated.[203]

Given the circumstances, we were surprised to observe rapid and selective enlargement of spines during our initial trials in 2001, achieved through two-photon uncaging.[128),204),205] This targeted enlargement was accomplished using a pairing protocol involving two-photon glutamate uncaging (60 times at 1 Hz) on a single spine, without the presence of extracellular Mg^{2+} or a depolarizing potential, to maximize NMDA receptor activation (Fig. 7A).[34),127] Repetitive uncaging beams were consistently positioned at least 0.3 µm distal to the spine in order to prevent direct irradiation onto the spine and mitigate the potential risk of damage.[128),206] We also noted similar enlargement by electrical stimulation of presynaptic fibers; however, the occurrence of spine enlargement was sporadic, becoming evident only with the knowledge gained from two-photon uncaging. The Hayashi lab also demonstrated spine enlargement and actin polymerization using focal electrical stimulation of presynaptic fibers,[207] although this method had the inadvertent capacity to directly depolarize dendrites.

The identification of spine enlargement in 95% of uncaged spines prompted us to explore into the functional alterations within synapses following this phenomenon. Two potential explanations were considered: first, spine enlargement might enhance postsynaptic glutamate sensitivity (P2); second, it might exert pressure on the presynaptic terminal, thereby boosting its function (P5) (Section 8.1.1). At this time, suitable methods for confirming the second possibility were lacking. However, we could investigate the first hypothesis through glutamate sensitivity mapping. This approach involved systematic two-photon glutamate uncaging before and after repetitive uncaging in perforated patched cells during depolarization to activate NMDA receptors. Perforated patching was chosen since we had observed that spine enlargement dissipated rapidly within 5 minutes of whole-cell perfusion, akin to LTP induction.[208),209] After two years of dedicated effort from 2003 to 2004, we successfully generated precise maps of glutamate sensitivity before and after inducing spine enlargement, facilitated by automated software. These maps revealed an augmentation of glutamate sensitivity upon inducing spine enlargement. Notably, the heightened glutamate sensitivity was localized at the specific hotspots, indicating a specific increase in glutamate receptors at the postsynaptic sites. Consequently, for the first time, we obtained evidence that supported Hebb's original proposition of synapse-selective associative plasticity (Section 4.1)[14] and its manifestation in dendritic spines.

Spine enlargement and the increased glutamate sensitivity were confirmed through two-photon uncaging experiments by other labs, including the Svoboda, Lisman, Sabatini, Yasuda, and Zito

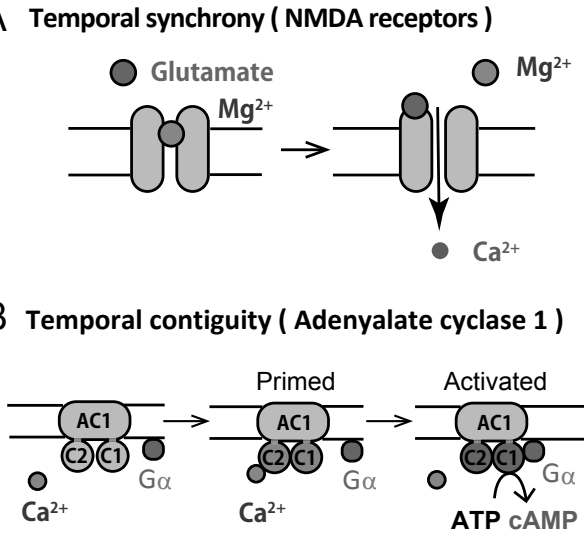

Fig. 7. (Color online) Two coincidence detectors for associative learning. (A) NMDA receptors for Hebbian associative learning. Two stimuli are applied simultaneously to induce Ca^{2+} influx through NMDA receptors. Glutamate activates NMDA receptors and membrane depolarization opens the gate for cation flux by removing Mg^{2+} from its pores. Membrane depolarization detects the presence of associative excitatory synaptic inputs. (B) Adenylate cyclase 1 (AC1) for the associative learning of Pavlov and Thorndike. Ca^{2+} influx through NMDA receptors occurs during conditioned sensory stimuli (CS) for Pavlovian conditioning and during the operant behavior of Thorndike RL. If such Ca^{2+} influx is transient, it primes AC1 for only 0.5–2 s after Ca^{2+}/calmodulin binding when AC1 is readily stimulated by Gs released from GPCRs (not shown). AC1 then produces cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). Such Gα release is induced by the activation of Gs-coupled GPCRs such as the D1 dopamine receptor. In the case of Gi,o-coupled GPCRs, such as the D2 dopamine receptor, the reduction of agonists deactivates Gi,o to disinhibit AC1. AC1 activation requires both $[Ca^{2+}]_i$ and G protein signals to act as detectors of temporal congruency. It is worth noting that this sequence of transitions is predicted from physiological investigations but has not yet been investigated in structural biology.

labs.[117),206),210)–214)] Spine enlargement showed both short-term (STE) and long-term (LTE) components (Fig. 2A, B), with glutamate sensitivity increases correlating more LTE volume increases than STE (Fig. 2B, C) (Section 8).[128),215),216)] The increases in glutamate sensitivity associated with spine enlargement is referred to as a form of general LTP, with LTE also recognized as structural LTP (sLTP).[217)] Smaller spines, often linked to silent synapses (Section 4.3), more effectively exhibited spine enlargement. This is due to their higher spine $[Ca^{2+}]_i$ from their smaller volume and significant NMDA receptors despite their smaller size (P1). Moreover, the greater resistance of the spine neck contributes to containing Ca^{2+} outflow.[112),218)]

Spine enlargement can also be induced using the chemical LTP protocol (cLTP), designed to minimize extrasynaptic NMDA receptors activation.[219)–221)] Recently, the Murakoshi lab introduced photoactivatable CaMKII (paCaMKII) as a useful tool for triggering spine enlargement and LTP.[215)] However, it is important to note that the resulting enlargement exhibits distinctive features compared to LTP and uncaging-induced LTE, possibly because of the differences in the spatiotemporal dynamics of CaMKII activation. Moreover, direct irradiation of the spine with a blue laser for photoactivation often leads to phototoxicity, making it advisable to evaluate the efficacy of inactive versions.

5.2. Unique transitions of spine enlargement.

5.2.1. Actin dynamics during early spine enlargement. Dynamic transitions of spine enlargement were investigated by Honkura in 2008, using two-photon photoactivation of a photoactivatable green fluorescent protein fused with actin to gain insight into the processes of spine enlargement. The initiation of spine enlargement involves the formation of a stable actin pool (enlargement pool) through actin polymerization.[135)] The significance of actin polymerization is evident from the partial rescue of whole-cell washout of spine enlargement by β-actin in the pipette.[152)] As actin fibers elongate, they generate an expansive force that enlarges the spine head and neck.[53)] Throughout this process, actin fibers become decorated with numerous other scaffolding and enzymatic proteins, forming a gel that can flow through the spine neck into the dendritic shaft. Approximately 50% of spines experience the flow of the actin gel, leading to widening of the neck.[135)] Subsequently, when the actin gel completely flows out, it generates a standalone STE whose duration varies from 5 to 20 min. The flow out of the enlarged pool and failure of LTE is facilitated by inhibition of calcium-calmodulin-dependent protein kinase CaMKII.[135)]

5.2.2. Transition to LTE. In response to the queries raised in Section 4.1 regarding LTP, the transition from spine transient enlargement (STE) to LTE involves the crucial participation of numerous molecules, which is vital for the stabilization of the enlargement process. Several mechanical factors come into play, including the size, stiffness, and adhesive properties of the actin fiber enlargement pool; diameter of the spine neck; and rigidity. These factors collectively impede the transit of the enlargement pool while stabilizing the enlargement process.[135)] Moreover, various molecules associated with subcellular structures, such as lamellipodia and stress fibers, also play a significant role in maintaining spine enlargement (further discussed in Section 5.3).[93)]

5.2.3. Uniqueness of electrode-induced LTP. LTP associated with LTE is a very specific phenomenon correlated with the enlargement of small spines,[222)] which represents a subset of the broader spectrum of structural plasticity observed in the spine (Fig. 4A). The comprehensive plasticity of spines encompasses various processes, such as the generation of new spines/filopodia, enlargement of large spines, neuromodulation (P3), slow fluctuations over weeks (P4) and pushing of presynaptic terminal (P5). Interestingly, the enlargement of spines may show a more rapid washout by whole-cell perfusion compared to LTP.[155),223),224)] A similar dissociation is observed when p38 mitogen-activated protein kinase (p38 MAPK) is blocked,[225)] indicating that not all instances of LTP are accompanied by LTE. The structure-function relationship (P1) suggests that when LTP does not coincide with structural changes, it may have a shorter duration, lack synapse specificity,[226)] and be less distinctive than spine enlargement. Conversely, it has been consistently observed that LTE is accompanied by an increase in glutamate sensitivity (P1; P2, Section 5.1).

5.2.4. Locus of LTP expression. Although spine enlargement is the predominant source of associative plasticity, the site of expression can vary in a time-dependent manner, as illustrated in Fig. 2E. The initial expansive force observed during spine enlargement matches the force during smooth muscle contraction, thus enabling rapid effects on presynaptic terminals in conjunction with spine expansion (P5). Furthermore, the late phase of LTE may engage in protein synthesis-dependent processes

along with an increase in PSD and presynaptic AZ,[153] likely enhancing presynaptic functions (Fig. 2E) (P1). Therefore, although spine enlargement is primarily a postsynaptic event, it can also yield presynaptic repercussions. The contribution of the site of expression can also depend on the induction protocol, such as the depolarization of postsynaptic cells,[227] which influences the activation of a type of voltage-gated Ca^{2+} channel.[228)–230] Theoretical optimization of pre- and postsynaptic plasticity sites has been proposed.[231] The most definitive method for studying postsynaptic sites is the measurement of glutamate-induced currents using two-photon glutamate uncaging. Presynaptic expression sites should be directly examined using glutamate sensors, such as iGluSnFR (outlined in Section 8.1) or eEOS, in the future.[232]

In this section, we broadly consider spine enlargement (P2) while reserving the discussion of rapid synaptic mechanics, memory, and cognitive functions for Section 8 (P5). This approach allows us to concentrate on the more general aspects of spine enlargement and its implications, while also exploring the specifics of rapid synaptic processes and their pertinence to memory and cognitive functions in a dedicated section.

5.3. Molecular and cellular basis of spine enlargement.

5.3.1. Molecular mechanisms. Spine enlargement, consistent with LTP,[233),234] involves the activation of NMDA receptors and an increase in $[Ca^{2+}]_i$, which triggers the activation of CaMKII.[128),235] CaMKII constitutes 10% (0.4 mM) of PSD proteins,[236] and its activation requires the removal of the autoinhibitory domain, which exposes the kinase domain for phosphorylation of various proteins, including CaMKII itself, through autophosphorylation. Autophosphorylation renders CaMKII independent of Ca^{2+}, resulting in prolonged activation, which is required for enlargement and LTP.[234] CaMKII also directly binds to specific proteins, such as NMDA receptors (NR2B) and Rac-GEF, leading to Rac1 and p21-activated kinase activation for actin polymerization.[213),237),238]

In fact, LTE can be blocked by mutating the kinase sites or by using inhibitors such as CN21, CN27, and AIP.[239),240] Although CaMKII activation, as recorded by the Förster resonance transfer (FRET) probe Camui, lasts only 1 minute,[212),241] evidence suggests that CaMKII is also involved in the constitutive regulation of spine synapses.[242)–244] In addition to CaMKII, the activation of PKA,[245] PKCα,[246] and TrKB is also required for LTE.[152),247] The force generated by spine enlargement (Section 8.1.4) creates a diffusive space that allows for more enzymatic reactions to occur.[248),249]

Protein synthesis-dependent enlargement is a crucial process in synaptic plasticity that can be induced by STDP.[152] Protein synthesis and BDNF play roles in LTE under various conditions[153),247),250] as well as in late-LTP.[251] This process is considered to be the most stable form of spine enlargement and likely forms the foundation of LTM.[252] The molecular pathway involved in protein-synthesis-dependent spine enlargement includes the activation of CaMKII, which leads to the disruption of SynGAP, a synaptic Ras-GTPase activating protein that is highly concentrated in excitatory synapses.[253] In turn, this activates an extracellular signal-regulated kinase (ERK, p41 MAPK) and subsequent protein synthesis via mammalian target of rapamycin signaling.[254] Protein synthesis and BDNF also play important roles in LTP under various conditions.[245),255),256] Furthermore, Ca^{2+}-induced degradation of RNA-induced silencing complex also plays a role.[257] Polyribosomes are elevated at the spine base and necks,[258] and many synaptic proteins, such as CaMKII, β-actin, PSD95, and AMPA receptors, are ready to be translated in the dendrites.[259]

Drawing on the principles of classical mechanics, specifically the law of "action" and "reaction", it can be inferred that the synaptic cleft and axon also generate force towards the enlarged spine to maintain equilibrium within the synaptic structure at each moment (Fig. 5). The strength of this force, comparable to smooth muscle contraction (0.5 kg f/cm^2, as discussed in Section 8.1.4) exerts a significant effect. Compliance with adhesion structures causes deformation of the synaptic cleft, subsequently signaling the spine. The axonal "reaction" impedes the growth of spine actin fibers towards the bouton opposing surface and influences spine structural plasticity. This force may also facilitate the trafficking of vesicles containing AMPA receptors and autocrine exocytosis of BDNF, stimulating specific molecules in the membrane. Furthermore, force generation creates new space for further enzymatic reactions.[248] Although the actions and reactions are often inseparable, they warrant careful consideration in future research. Dendritic spines serve as force generators that affect all molecules in their mechanical environment. Therefore, spine enlargement represents a core mechanical process that impacts both postsynaptic spine itself (P2) and its presynaptic terminal (P5).

5.3.2. Why is spine enlargement specific to stimulated spines? The selective enlargement of stimulated spines is a hallmark of this synaptic plasticity. However, the reason for the confining structural plasticity is not straightforward, as nearly all molecules in the spine are involved, and they all diffuse along the dendrites to some degree when not engaged in spine enlargement. For instance, some upstream molecules, such as Ca^{2+}, CaMKII, and small GTPases, are localized to spines within 2–15 µm from the stimulated spines.[235] On the other hand, a major effector of spine enlargement, phosphorylated cofilins, can readily spread along dendrites.[260] Consequently, the confinement of enlargement is only understood through the formation of high-order molecular structures, such as filopodia, lamellipodia and stress-fibers,[6] similar to living dissipative structures like finger prints, irises, or facial features, which are used as personal identifiers.[261] Within these molecular arrangements, even as the constituent molecules undergo turnover and dispersion, the original structural integrity persists. It is worth noting that distant propagation of LTP has been reported at the synapses without spines in the hippocampus.[226]

5.3.3. LTP: Capture of AMPA receptors by spine enlargement. The selective accumulation of AMPA receptors is responsible for the enhancement of glutamate responses at the synapse.[163),209),262)–264)] This accumulation can occur through various mechanisms, resulting in the establishment of a stationary structure-function relationship (P1) (Section 4.4). Firstly, extracellular AMPA receptors are abundant,[158),164),265)] can diffuse laterally,[266)] and become trapped by auxiliary subunits of AMPA receptors in PSD proteins (Section 4.4). Prevention of lateral diffusion of GluA2 by cross-linking impairs the potentiation of GluA2.[124),264)] Secondly, the Ca^{2+}-dependent exocytotic insertion of the receptors via unique sets of synaptotagmins and soluble N-maleimide-sensitive fusion attachment *protein* receptor (SNARE) proteins,[267),268)] supplies the pool of AMPA receptors.[269),270)] The time course of exocytosis may be slower than that of lateral diffusion.[271)] Thirdly, CaMKII-mediated phosphorylation of AMPA receptor[272)] and TARPs[273)–275)] may facilitate the accumulation of AMPA receptors to PSD.[276)] Despite the existence of several molecular mechanisms for the accumulation of receptors on enlarged spines, this accumulation is slow (5–10 minutes).[128),206),216),217),263),271)] Thus, actin-driven spine enlargement tends to precede the accumulation of AMPA receptors.

5.3.4. Hippocampus, neocortex, amygdala, and striatum. LTP has been reported in various parts of the brain, including the hippocampus, neocortex, amygdala, striatum, and spinal cord,[277)–280)] which is consistent with learning-induced spine generation and enlargement occurring *in vivo*.[50),123),252),281)–288)] These observations likely reflect the enlargement and stabilization of small spines (Fig. 4A). The critical role of CaMKII in spine enlargement and generation has been highlighted in studies of the autophosphorylation mutant of CaMKII (CaMKII-T286A) knock-in mice, which lack these processes.[289),290)] However, the neocortex is generally a slower learner, and two-photon uncaging can induce spine enlargement to a lesser degree and has a lower incidence than in the hippocampus.[155),260)] Individual steps of enlargement in the neocortex may be often pose a challenge to detection using current microscopic methods despite their critical role. In addition, there is protection against overwriting, as discussed in the next section. The neocortex requires hippocampal replay to establish recent memories, whereas the hippocampus exhibits more frequent and rapid spine enlargement, which is consistent with its role in the storage of new memories. The ventral striatum's highly plastic nature of spine synapses makes it more adept at acquiring appetitive conditioned learning.[70),110)]

5.3.5. Variability of spine enlargement and cascade model. One aspect of single-spine analysis is the degree of variability in spine enlargement from one spine to the next (see for a typical example, Extended Data Fig. 6 of Ref. 247).[128),211),260)] Although this variability may initially seem a problem, it could actually represent the internal state of spines protected from activity-dependent plasticity. This assumption is similar to the cascade models of synapses,[291)] where each synapse has connectivity together with meta-plasticity mechanisms that protect memories from overwriting, may increase memory capacity almost linearly with the number of synapses N, and undergo approximately $(1/\sqrt{t})$ decay followed by an exponential decay (Section 7.2.3).[292)]

The observed variability in spine enlargement may be attributed to high levels of variability in the expression levels of key proteins at the cellular level, such as CaMKII,[293)] and even among spines for PSD95 and SAP102.[294),295)] This variability in PSD protein expression is present throughout the brain.[296)] Other contributing factors are the saturation of LTP[297)–299)] and enlargement[300)] and the invasion of the endoplasmic reticulum.[52)] However,

when considering realistic networks, the cascade model should incorporate the intrinsic dynamics of spines (P4) (Section 7.2.4).

5.4. Activity-dependent spine shrinkage.

5.4.1. Background. Finding stable induction protocols for LTD has been challenging because it requires precise setting of global $[Ca^{2+}]_i$ to an intermediate level.[301),302)] In the 1990s, a low-frequency repetitive stimulation paradigm was discovered (1 Hz, 900 times),[277),303)] followed by the t-LTD paradigm of STDP in 1998 in an NMDA receptor-dependent manner.[304)–307)] Similar to LTP, LTD is pathway specific, $[Ca^{2+}]_i$-dependent, and has an association with spine structures.[311)] However, LTD showcases asymmetry to LTP in various facets. Firstly, LTD depends on protein phosphatase calcineurin, which dephosphorylates cofilin, rather than CaMKII.[92),301),308)–312)] Secondly, GABA inhibition weakens LTP,[313),314)] but augments LTD.[315)–318)] Thirdly, LTD develops more gradually up to 30 minutes, in contrast to the rapid onset of LTP.[92),319)–321)] Fourthly, LTD has the pathway selectivity that was defined by using two electrodes in previous studies (Fig. 6A),[311)] but the notable spread of LTD has also been reported.[318),322),323)] Finally, LTD is often hetero-synaptically induced surrounding LTP induction sites.[311),324)–327)]

5.4.2. Unveiling our discovery of spine shrinkage. Spine shrinkage was first reported by LTD induction protocols using electrical stimulation of presynaptic fiber bundles in the Poo lab in 2004.[312),328)] However, spines that were stimulated and whose function changed were not identified, and shrinkage may have been due to the stimulation of other spines. To clarify this issue, single spines must be stimulated to induce spine shrinkage. Hayama used two-photon glutamate uncaging in 2008 and found that STDP with LTD-timing (post-pre spikes) did not induce spine shrinkage, although spine enlargement could be induced by LTP-timing (pre-post spikes).[152)] We speculated that there was an additional factor necessary to induce spine shrinkage. A previous study reported the key role played by GABAergic input in hippocampal LTD in 2010, as electrodes stimulate GABAergic fibers (Fig. 6A).[318)] At that time, we developed a two-photon-compatible GABA[329),330)] and experimented with using two caged compounds simultaneously. The Yuste lab developed RuBi-GABA, which can be activated by blue light at 458 nm with little two-photon absorption, showing the best combination with CDNI-glutamate. Thus, we applied STDP with t-LTD timing, in which GABA uncaging was performed in a nearby dendritic shaft. This was the first experiment in which we observed large shrinkage and even pruning of the stimulated spine. Our study demonstrated that spine shrinkage could reach up to 100% (elimination) and on average 50%,[109)] similar to classical LTD.[311)]

5.4.3. Spine shrinkage underlying LTD. The spine shrinkage induced by single-spine glutamate uncaging fulfils the five features of LTD listed above. Using the t-LTD timing of STDPs, the strong spine shrinkage and pruning was found in NMDA receptors, calcineurin, and in a cofilin-dependent manner,[109)] which support the findings of a previous experiment.[312)] The spine shrinkage is abolished by Ca^{2+} buffers, and it is associated with a decline in glutamate sensitivity when spine shrinkage occurs.[109)] GABA uncaging could be replaced with GABA (0.2 µM) in the bathing solution, but not when presynaptic glutamate fibers are selectively optogenetically stimulated.[331)] Furthermore, spine shrinkage can be induced *in vivo* under the presence of GABA agonists.[260)]

GABA facilitates spine shrinkage and LTD, likely through modulation of intracellular Ca^{2+} levels, because GABA can be replaced with a slow Ca^{2+} buffer, EGTA (3 mM) (Fig. 8A–C).[109)] Importantly, in adult animals or in CA3 pyramidal neurons, the same timing that induces LTD (pre-post spikes) can instead produce LTP due to high $[Ca^{2+}]_i$ increases (Fig. 8D).[332),333)] A similar mechanism may apply to the symmetric time window for behavioral time scale plasticity (BTSP) found by the Magee lab (Section 6.5.1),[334)–336)] dendritic distance dependence of STDP,[320),321)] and GABA-dependence of critical period plasticity (Section 5.5). Thus, the increase in bulk $[Ca^{2+}]_i$ levels determines the direction of plasticity by CaMKII activation.[301),302),337),338)] The Bito lab clarified that calcineurin is activated in the conditions where CaMKII is activated (Fig. 8).[339)] However, many actions of calcineurin, if not all,[340)] are prevented by CaMKII (Fig. 1c of Ref. 6), where CaMKII is concentrated ten times more than calcineurin in spines.[236)]

Spine shrinkage also requires a Ca^{2+} domain because a fast buffer, BAPTA, cannot replace GABA (Fig. 8A, B),[109)] similar to spine enlargement[212)] and presynaptic neurotransmitter release.[341),342)] These results indicate that the Ca^{2+} domain of NMDA receptors can be used to precisely detect the presence and location of synaptic input. Without this specific Ca^{2+} domain, plasticity may be induced by a small

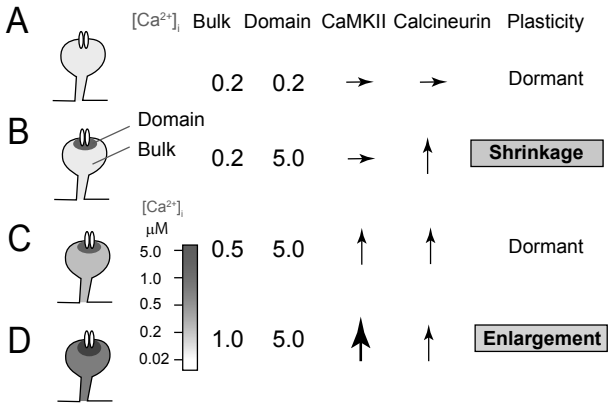

Fig. 8. (Color online) Differential induction of spine enlargement and shrinkage, and involvement of the Ca^{2+} domain in both cases. Increase in cytosolic bulk and domain Ca^{2+} concentration. In the bulk phase, Ca^{2+} equilibrates with cytosolic Ca^{2+} buffer. In contrast, Ca^{2+} influx through Ca^{2+} channels and NMDA receptors occurs at the inner opening of channels and activates adjacent Ca^{2+}-binding proteins before binding to other cytosolic Ca^{2+}-binding molecules. This Ca^{2+} domain effect is suitable for the rapid focal activation of target molecules and is used to detect postsynaptic activation of NMDA receptors. (A) Without domain-phase activation, neither enlargement nor shrinkage is induced.[109),212)] The values of $[Ca^{2+}]_i$ are an example. (B) Induction of spine shrinkage by a low-bulk $[Ca^{2+}]_i$ increase and Ca^{2+} domain. Calcineurin, a major cytosolic protein, has a high-affinity Ca^{2+}-binding site and a low-affinity Ca^{2+}/calmodulin-binding site for the detection of synaptic inputs and weak depolarization. The major mechanism of action of calcineurin is cofilin activation via dephosphorylation. (C) No structural changes in the spine with higher bulk $[Ca^{2+}]_i$ increase. Under these conditions, CaMKII is activated, which inhibits calcineurin activity. Spine shrinkage can be induced when bulk $[Ca^{2+}]_i$ increases are suppressed by extracellular GABA or intracellular Ca^{2+} buffers (Section 5.4.3). (D) Spine enlargement owing to a larger increase in bulk $[Ca^{2+}]_i$ and Ca^{2+} domains. Under these conditions, both CaMKII and calcineurin are activated. CaMKII concentration in postsynaptic spines is very high (0.4 mM), which impedes calcineurin activity. The resulting major actions are the activation of Rho-family GTPases and phosphorylation of cofilin by CaMKII. This figure is a modified version of the Hayama *et al.* (2013).[109)]

increase in dendritic $[Ca^{2+}]_i$ due to low-frequency spikes (Fig. 8A). These findings suggest that both the medium level of bulk $[Ca^{2+}]_i$ and high level of $[Ca^{2+}]_i$ increase (Ca^{2+} domain) near NMDA receptors are necessary for spine shrinkage.[109)] We did not find any fine structural changes in the state depicted in Fig. 8C, demonstrating the remarkable integrity of spine function.

Spine shrinkage, which reflects a reduction in the size of the PSD, occurs gradually with a time constant of approximately 10 minutes in the hippocampus,[109),225)] and in the adult neocortex.[343)] It appears that activated cofilin severs F-actin, eventually leading to a reduction in PSD size. Cytochalasin, which binds to F-actin and blocks its polymerization, disrupts the PSD95 cluster.[344)] Latrunculin-A, which binds with G-actin and block polymerization, has been shown to cause a slow reduction of PSD95.[344),345)]

5.4.4. Pathway specificity of LTD and spine shrinkage. To our surprise, when spine shrinkage was induced to the extent that it triggered spine pruning in the stimulated spine, the pruning spread up to 7 μm from the site of stimulation.[109)] Even with more moderate spine shrinkage, it spread to neighboring spines up to 15 μm in the hippocampus,[109)] and 3 μm in the neocortex.[260)] This finding does not contradict the pathway specificity of LTD, because LTD was induced using a metal electrode that stimulated presynaptic fiber bundles (Section 4.1). The separation between the two stimulation electrodes that were placed on opposite sides of the recording cells (Fig. 6A) could be greater than 15 μm, making the spread impossible to detect. Importantly, we found that spine enlargement can resist spreading spine shrinkage,[109)] preserving independent modifiability. Therefore, shrinkage occurs competitively with spine enlargement (Section 5.5). Finally, massive spine enlargement induces shrinkage of adjacent spines,[346)] similar to the spread of LTD surrounding LTP (Section 5.4.1). Thus, spine shrinkage exhibits many features similar to those of LTD (Section 5.4.1).

5.4.5. Spine shrinkage due to the non-ionotropic action of NMDA receptors. Experiments conducted by the Malinow lab demonstrated that LTD can occur as a result of the non-ionotropic (metabotropic) action of NMDA receptors. Their findings revealed that LTD was blocked by the NMDA receptor antagonist 2-Amino-5-phosphonopentanoic acid, but not by an open channel inhibitor MK801 or a glycine site antagonist 7-chlorokynurenate.[347)] Similarly, the Zito lab showed that non-ionotropic actions of NMDA receptors could induce spine shrinkage and LTD when low-frequency stimulation was applied together in conjunction with long depolarization,[348)] or when the glycine site of NMDA receptors was blocked.[349)–351)] Traditional LTD and Ca^{2+}-dependent spine shrinkage show approximately 50% shrinkage (Section 5.4.2); however, the type of shrinkage induced by non-ionotropic NMDA receptor actions results in approximately 25% shrinkage.[348),349)] This type of shrinkage differs from classical studies where LTD and shrinkage were

blocked by cytosolic Ca^{2+} buffers.[109),310),352)] In addition, the shrinkage induced by non-ionotropic NMDA receptor actions was found to be eliminated by the activation of p38 MAPK.[225)] Furthermore, this form of spine shrinkage was independent of metabotropic glutamate receptors.[348),349)] It is important to note that this type of LTD may occur under specific circumstances, such as lower PSD95 expression; in certain disease states[353)]; or when the glycine site is blocked.[349)] Moreover, it may coexist with the classical forms of LTD and spine shrinkage (Sections 5.4.1–5.4.4). Nevertheless, despite these distinctions, a phenomenological parallelism between LTD and spine shrinkage is observed at this level.

5.4.6. Spine plasticity in metabotropic glutamate receptor-dependent LTD. Structural changes in spines in response to metabotropic glutamate receptor (mGluR)-mediated LTD have been investigated in cerebellar Purkinje cells[354),355)] and hippocampal pyramidal cells.[356),357)] Interestingly, these studies demonstrated that mGluR-dependent LTD does not necessarily result in spine shrinkage. This observation is in line with the structure-function relationship (P1), suggesting that spine structure must eventually equilibrate with spine function. Therefore, synaptic plasticity without a structural basis may not last long. Indeed, although mGluR dependent LTD is known to be induced in Purkinje cells for motor learning,[358),359)] it is considered that the memory is transferred from the cerebellar cortex to the cerebellar nuclei for long-term storage.[360),361)] However, the considerable amount of information retained in the cerebellar cortex cannot be directly transferred to the cerebellar nuclei. Consequently, to foster learning that requires precise timing and coordination—often seen in rhythmic or metrical tasks—as well as adaptive learning, regular training may be essential.

5.4.7. Asymmetry between spine enlargement (or LTP) and shrinkage (or LTD). As discussed in Sections 5.2 and 5.3, spine enlargement (or LTP), and spine shrinkage (or LTD) exhibit asymmetry in their kinetics and spatial spread due to their biochemical bases. Spine enlargement occurs rapidly within 10 seconds,[128)] primarily due to actin reorganization followed by gradual enlargement of the PSD, whereas spine shrinkage develops gradually within 10–30 minutes mainly due to a slow reduction in PSD. Moreover, spine enlargement can be synapse-specific owing to the specific actin scaffold, whereas spine shrinkage tends to spread by shrink factors, such as cofilin, although it still maintains the pathway specificity of LTD studies with field EPSPs. Consequently, their physiological roles are also asymmetric: rapid and synapse-specific enlargement (LTP) is more suited for online memory storage, whereas shrinkage (LTD) is better suited for offline removal of inappropriate memories by removing synapses that were not stimulated in a Hebbian manner.[109)] Although spine shrinkage with the pathway specificity of LTD can act as shaping memory[362)] and working memory, it does not act as fear conditioning and spatial reference memory when impaired in calcineurin knockout mice[363)] who have a severe deficit of LTD.[364)] The meanings of this asymmetry in learning are underexplored even in neural network models.[6)]

5.5. Clustering of dendritic spines and GABA-mediated synaptic competition. *In vivo* two-photon investigations of spine dynamics have revealed that the sensory experience mediated by whisker stimulation selectively potentiates nearby spines in the mouse barrel cortex by promoting AMPAR insertion.[365)] In contrast to baseline conditions, where new spines appear to avoid existing stable spines on the same dendritic branch, neighboring spines tend to appear as a pair of spines in trained mice reaching through a slit to grasp a food pellet.[366)] The spatial clustering of inhibitory synapses and dendritic spine remodeling within 10 µm is influenced by sensory input during normal visual experience.[367)] Hotspots of spine turnover in the retrosplenial cortex predict future memory performance.[368)] This clustering may be one way in which similar inputs can survive in nearby dendrites.[285)] Neighboring spines tend to receive similar synaptic inputs.[369),370)] Clustering of synaptic inputs significantly impacts dendritic excitability as adjacent inputs can nonlinearly summate,[371)–373)] making each dendritic compartment an independent summing unit and significantly increasing information storage capacity, allowing a single neuron to behave as an abstract neural network.[196)] Clustering of spines is advantageous for eliciting local action potentials for information transmission, possibly due to the reduced threshold for spine enlargement resulting from the spread of activation of the Ras and Rho family proteins, ERK and PKA over 10 µm.[206),211),213),245),247),374)]

GABA interneurons can induce branch-specific alteration of dendritic spines.[375)] Activation of GABA neurons facilitate in the elimination different conditionings on the same dendritic branches, and promote fear extinction.[376)] Spine pruning is promoted during the critical period in a GABA-dependent manner,[377)] consistent with the GABA-depend-

ence of spine shrinkage and elimination as discussed in Section 5.4.2.[109] The spread of spine shrinkage (non-Hebbian) contributes to the removal of useless synapses that can be replaced with useful synapses for synaptic reorganization during the critical period. Although the spread of spine elimination is not evident in *in vivo* time-lapse imaging, owing to the spontaneous generation of spines (Section 7.1), both spine enlargement (genesis) and shrinkage (pruning) are used for the competitive selection of better synapses and circuits.

6. Time-dependent modulation of extrinsic dynamics by GPCRs (P3: Neuromodulation)

6.1. A brief history of conditioning and RL. In the early 20th century, Pavlov noticed that when a tone was shortly followed by the presentation of food, dogs started to salivate in response to the tone alone, which is now known as classical conditioning.[55] Similarly, Thorndike found that certain cat behaviors could reinforced when shortly followed by a food reward, leading to the development of the concept of operant conditioning.[54] Since then, numerous human[378),379] and animal studies[380),381] have demonstrated that reward timing is most effective within a short time window of approximately 0.5–2 seconds. Within this timeframe, animals interpret the congruency between behaviors and rewards as contingent and assign a positive value to the corresponding stimuli or behaviors. Rescorla proposed that the reinforcer is actually the difference between the predicted reward and actual reward,[380] also known as reward prediction error (RPE).[382),383] Reward and punishments may also originate from internal events, with positive and negative emotions being formed through learning across an individual's lifetime via both external and internal events.[384]

The relatively slow timing of the reward posed a challenge in explaining it solely through the tens of milliseconds of synaptic associations formed by NMDA receptors (Fig. 8A). Thus, whether reward timing arises from synaptic or circuit mechanisms had been a matter of debate until recently.[385),386] In machine learning, the "credit assignment problem" involves identifying specific states or actions that are associated with each reward.[38] In the case of conditioning, sensorimotor stimuli leave an "eligibility trace" in the brain, which helps in understanding conditioning by identifying specific states or actions associated with each reward in a similar way as in machine learning.

Brain self-stimulation studies have identified the midbrain dopamine neurons and their primary projection site, the striatum, as crucial regions for self-motivation. Many addictive drugs such as cocaine and methamphetamine (MAP) have been found to increase dopamine concentrations in the brain. It has also been observed that dopamine neuron firing increases during water rewards in monkeys, particularly before conditioning, and decreases after conditioning has been established, in accordance with RPE signals.[382),387),388] Dopamine serves as an agonist for hundreds of GPCRs, which regulate key enzymes in the plasma membrane, including adenylate cyclase (AC). Upon agonist binding, AC generates the second messenger, cyclic adenosine monophosphate (cAMP), in the cytosol (Fig. 7B). Notably, the nucleus accumbens (ventral striatum, NAc) is considered a critical brain region for reward conditioning,[389),390] and dopamine fiber stimulation has been shown to facilitate reward-related synaptic plasticity between the cortex and striatum.[391] It is highly plausible that the striatum implements an RL algorithm using dopamine as an RPE signal. Recently, the nature of the biological eligibility trace for conditioning was identified.

6.2. Unveiling our discovery of the biological basis of eligibility trace in the striatum.

6.2.1. D1 neurons and eligibility traces. In the striatum, D1 and D2 neurons are the two main types of spiny projection (medium spiny) neurons.[392] These neurons receive strong glutamatergic inputs from the cortex and thalamus on their dendritic spines, which exhibit similar shapes and densities to cortical neurons.[1] The former express D1 receptors, whereas the latter express D2 receptors, and both receive the densest dopaminergic innervation from the ventral tegmental area (VTA). However, investigating the time-dependent effects of each type of input has been challenging because of the technical difficulty in selectively stimulating dopaminergic or glutamatergic fibers without activating the other (Fig. 6A). Previous studies were unable to dissociate the specific contributions of these two inputs to the modulation of D1 and D2 neurons.[393] To address this issue, Yagishita *et al.* employed a novel approach to examine the role of dopaminergic inputs in reward timing. Specifically, they used slice preparations from 4-week-old dopamine transporter (DAT)-Cre mice expressing the channel rhodopsin2 (ChR2) in the VTA. Using this technique, dopamine fibers can be selectively stimulated with blue light, whereas glutamate receptors can be activated by two-

photon glutamate uncaging. By using this approach, they were able to dissociate the effects of dopaminergic and glutamatergic inputs on the modulation of D1 and D2 neurons in the ventral striatum (NAc).[110]

In D1 neurons located in the NAc, dopamine induces spine enlargement in the presence of STDP. To investigate the temporal dynamics of dopamine signaling, optogenetic burst stimulation was applied to dopaminergic fibers at various times relative to STDP, and paired STDP and dopamine bursts were repeated 15 times (Fig. 3A, B). Surprisingly, the results showed that paired STDP and dopamine stimulation were effective only if dopamine was delivered after STDP, with delays ranging from 0.3 to 2 seconds. This suggests that dopamine is only required for spine enlargement during this narrow time window (Figs. 3 and 6). The short timing has been confirmed in another study.[394]

6.2.2. Molecular basis of the eligibility trace and its formation. The molecular basis of the eligibility trace and its formation have been investigated using FRET sensors for CaMKII and PKA activation, Camui[395] and AKAR,[396),397] respectively. The timing of spine enlargement replicated the time course of CaMKII and PKA activation. Specifically, sensory stimuli increased $[Ca^{2+}]_i$ and primed AC1, a Ca^{2+}-dependent adenylyl cyclase that generates cAMP when dopamine arrives shortly after (Fig. 3A, 7B). Indeed, the effects of dopamine can be blocked by an inhibitor of the Ca^{2+}-dependent subtype of AC, AC1. cAMP activates PKA, which, in turn, facilitates Ca^{2+}-induced CaMKII activation. Accumulation of cAMP during repetitive pairing of STDP and dopamine, which was performed 15 times, led to spine enlargement (Fig. 3). A more detailed kinetic explanation of this process has been provided elsewhere.[398] Interestingly, although CaMKII activation is confined to the stimulated spine, PKA activation spreads over a distance of 10 µm,[110),245] as discussed in Section 6.4.3.

The timing of the eligibility trace observed at the synaptic level in striatal D1 neurons is consistent with the reward timing observed in head-restrained mice conditioned with tone and water.[381] This timing is also in line with conditioning tasks performed in other animals and humans. Interestingly, similar mechanisms have been proposed for the presynaptic terminal of the Aplysia siphon withdrawal reflex, which is conditioned by tail shock. The same time window and mechanism for Ca^{2+}-dependent AC have been suggested.[399)–401] Although the experimental paradigms used in these studies are not identical to those of classical conditioning because CS stimuli already induces a reflex strengthened by conditioning,[399] AC1 appears to be used in a similar manner in presynaptic terminals. These findings suggest that the fundamental timing generator for conditioning may be AC1, and that the atomic structure of AC1 may be involved in the formation of the eligibility trace (Fig. 7B).

6.2.3. AC5 in the striatum. AC5 is a Ca^{2+}-insensitive adenylyl-cyclase subtype that is considered a major subtype in striatal physiology.[402)–404] Therefore, it has been challenging to speculate on the specific function of Ca^{2+}-sensitive AC1 in the striatum. AC5 appears to play a role in somas, whereas the fine dendritic processes of striatal neurons are advantageous for the rapid Ca^{2+} kinetics required for conditioning mediated by AC1. Notably, somatic cAMP may be involved in the expression of emotional behaviors by increasing excitability, whereas dendritic cAMP signaling mediated by AC1 is more likely to underlie synaptic plasticity and learning.[405),406] Therefore, the distinct localization and function of AC1 and AC5 suggest that they play complementary roles in the regulation of striatal physiology and behavior.[398]

6.3. Modulation of spine enlargement by GPCRs coupled with Gi,o.

6.3.1. D2 neurons and psychosis. Antagonists of D2 receptors (D2Rs) are commonly used in clinical practice as antipsychotic medications and major tranquilizers. By inhibiting D2R (D3R, D4R), these drugs suppress Gi,o signaling and generally activate D2 neurons, leading to the suppression of cortical activity via an indirect pathway in the basal ganglia. In extreme cases, the activation of all D2 neurons in the NAc can induce slow-wave sleep in mice.[407] Under physiological conditions, the selective activation of D2 neurons specifically suppresses frontal cortical function. Psychosis, which is characterized by delusions and hallucinations, is a diagnostic symptom of schizophrenia that can be slowly mitigated by antipsychotic drugs. Impairment of D2R-related punishment-learning may contribute to the development of psychosis. Studies have suggested that the cessation of dopamine burst firing (dopamine dip or DA-dip) at a particular time triggers punishment learning.[382),387),388] However, this plasticity was not demonstrated until a recent study.

6.3.2. Unveiling our discovery of dichotomous modulation in slices. To investigate the effects of

DA-dips on spine enlargement in D2 neurons, Iino *et al.* generated various durations of DA-dips by inserting a pause during 5 Hz optogenetic dopamine fiber stimulation in slices.[70] An adenosine A2A receptor agonist was applied to activate Gs signaling in D2 neurons (Fig. 3C). Spine enlargement was induced when STDP coincided with the dip; however, there was no enlargement in the absence of an A2A receptor agonist or when there was no dopamine dip. Furthermore, enlargement was blocked by PKA and CaMKII inhibitors. Notably, the pause of the dopamine burst could be as short as 0.4 s and still be effective, which is consistent with the *in vivo* measurements of dopamine dips. D2Rs have a high affinity to dopamine (0.1 µM) and must be constitutively activated by 0.5 µM of dopamine at the resting 5 Hz firing rate of dopamine neurons.[408] In contrast, D1Rs have a lower affinity for dopamine, and D1 neurons do not display spine enlargement at resting firing levels. However, spine enlargement in D1 neurons could be induced if a 20 Hz burst was applied, yielding a dopamine concentration of 2 µM. Therefore, the modulation of synaptic plasticity by dopamine in D1 and D2 neurons is dichotomous, with dopamine selectively modulating D1 and D2 neurons at high and low concentrations, respectively.

6.3.3. Dichotomous modulation in vivo. Although it is well established that dopamine bursts initiate reward conditioning, the involvement of dopamine dips in punishment-learning remains unclear. To investigate this issue, Sawada *et al.* trained mice in a reward-conditioning task in which tone 1 signaled the availability of a water reward, whereas tone 2 did not. Within 10 trials, the mice showed licking responses to tone 1 but not to tone 2.[70] During this time, the dopamine concentration in the NAc increased during the reward period following tone 1, but showed a dip during the reward omission period following tone 2, which was consistent with previous studies.[382),387),388] They investigated the role of dopamine dips in discrimination learning by optogenetically stimulating dopamine neurons in the VTA to ablate dopamine dips. They found that the discriminatory learning was abrogated. The same impairment was observed when a CaMKII inhibitor (AIP) was selectively expressed in D2 neurons in the NAc or when an antagonist of the A2A receptor was bilaterally perfused into the NAc. Therefore, it is likely that tone 2, followed by dopamine dip-induced spine enlargement in D2 neurons, suppresses licking responses. Notably, dopamine dip detection also occurs following STDP within a few seconds, similar to the reward conditioning paradigm, and likely involves AC1.[409]

The dichotomous regulation of SPNs has also been observed *in vivo* using a PKA sensor, which showed that dopamine bursts activate PKA in D1 neurons, whereas dopamine dips activate PKA in D2 neurons.[406] These fiber photometry experiments suggest that Ca^{2+}-independent AC5 activity in the soma is the primary target of dopamine dips.

6.3.4. Psychosis. Repetitive administration of MAP, a known inducer of psychosis in humans and animals, has been shown to block spine enlargement in D2 neurons and impair discrimination learning. In mice, this was accompanied by hyperlocomotion, suggesting that psychosis arises from the impairment of learning in D2 neurons, which corrects improper conditioning, in line with the salience misattribution hypothesis.[410] Interestingly, our findings suggest that D1R-mediated reward learning may be generalized.[70] As a result, the efficacy of antipsychotic drugs may be attributed to the D2R-mediated removal of improper generalized associations, or imaginal associations, caused by D1R-mediated generalized reward learning. We reported a theoretical validation of our hypothesis.[411]

The occurrence of psychotic symptoms in patients with schizophrenia is a notable common phenomenon that occurs despite variations in genetic and environmental backgrounds. Two potential mechanisms may explain this phenomenon. The first is the hyper-dopamine hypothesis, which proposes that environmental stressors, particularly during adolescence, can result in overstimulation of the dopamine system. This can lead to abnormally high concentrations of dopamine in the NAc, impairing learning in D2 neurons.[410] It has been observed that drugs, such as NMDA receptor inhibitors, PCP, dopamine uptake inhibitors, and MAP, which increase dopamine concentrations, can induce psychosis and schizophrenia. Therefore, it is likely that psychosis can lead to further cognitive symptoms, including the impairment of plasticity. The second possibility is the impairment of general plasticity mechanisms as reported in genetic linkage studies, which includes D2 receptors, GluA1, NR2A, CaMKII, calcineurin, Ca^{2+} channels, phosphodiesterase, and regulator of G-protein (RGS) (Fig. 3C, red). The D2 signaling cascade, which regulates spine enlargement, contains additional G-protein-coupled regulatory mechanisms that are more complex than D1 signaling (Fig. 3C, red). Because D2R-dependent

learning requires the detection of a slight dopamine dip, the kinetics of the D2 receptor, G-proteins, RGS, and AC1, as well as downstream plasticity mechanisms, need to be well-tuned. Consequently, it is possible that learning involving D2R mechanisms is more vulnerable, leading to psychosis in the ventral striatum and dystonia in the dorsal striatum.[409] This possibility may provide an inherent explanation of hyper-dopamine state, as the reduced activity of D2 neurons could result in general increases in dopamine neuron firing.[411]

6.4. Relationship between dopamine modulation and RL theory. The modulation of spine synapses by dopamine supports the concept of RL theory because dopamine encodes RPE.[412),413] Moreover, the use of dopamine bursts and dips for positive and negative RL learning further supports this idea.[70] Nonetheless, it is important to acknowledge that there are distinct differences between machine learning-based RL and the biological mechanisms underlying dopamine modulation.

6.4.1. Dichotomous circuits. Dichotomous circuits play a crucial role in biological conditioning, as reward and punishment learning rely on two distinct pathways, exemplified by the dopamine D1 and D2 systems in the striatum (Fig. 3). Positive and negative memories are stored separately in distinct memory systems, which allows them to be independently evaluated and retrieved. Moreover, these memories can occur with similar time courses, as they both use spine enlargement (Section 5.3.6). In dichotomous circuits, the relative weighting of these memories can change in specific emotional states under the influence of different neuromodulators, leading to biased valuation and complex interactions between the two pathways. Consequently, RL becomes a more intricate optimization problem heavily influenced by neuromodulatory states. Further investigation of these processes is required to enhance our understanding of emotions and emotional disorders, including positive and negative (depressive) symptoms. One hypothesis is that dichotomous circuits accelerate and decelerate cortical structural plasticity to engender pleasant and unpleasant emotions, respectively.

6.4.2. Asymmetry in the positive and negative RPE signals. In RL in machine learning, symmetric positive and negative RPE signals can result in an increase or decrease in the value of states or actions, which correlates with the potentiation and depression of weights in the cortico-striatal pathway in the striatum.[38),382] However, there has been no successful induction of spine shrinkage in D1 and D2 neurons. LTD is presynaptic in spiny neurons and is mediated by endocannabinoids released from postsynaptic neurons.[393),414)–416] Most experiments have recorded LTD for up to 1 hour. If presynaptic LTD accumulates while postsynaptic LTP accumulates, the presence of positive and negative RPE signals could lead to a significant imbalance in synaptic function, which would be unrealistic. Moreover, even if spine shrinkage could be induced, it tended to exhibit a slower time course (Section 5.4.7); therefore, it cannot serve as a symmetric form of plasticity. Lastly, detecting dopamine dip and burst signals by D1 and D2 neurons, respectively, is challenging due to the affinities of dopamine receptors.[408]

Thus, it is noteworthy that spiny neurons rely solely on spine enlargement for conditioning; however, this does not result in spine size saturation because of the intrinsic dynamics of spines (P4) (Section 7.1). The spine structures in these neurons bear a close resemblance to those found in the cortex,[1),417] indicating that learning can be stably maintained without activity-dependent spine shrinkage and LTD.

6.4.3. Spread of RPE signals and eligibility traces along dendrites. In machine RL, the eligibility trace is specific to the internal state.[38] However, in the presence of conditioning, dopamine release in striatal spiny projection neurons causes the cAMP/PKA signal to propagate along dendrites for more than 13 μm and for a duration of 1 minute, rather than being restricted to the stimulated spine.[110),245] This results in an eligibility trace being created in the surrounding spines, potentially inducing generalization. To our knowledge, no RL model has been developed to account for this phenomenon yet.

6.4.4. Dependence of RPE on behavioral states and brain regions. Our results suggest that dopamine dips are detected in the reward omission task, but not in the extinction task, where the reward is consistently omitted. Although the dopamine hypothesis of the RL theory predicts that dopamine dips should occur in both tasks, animals can differentiate between discrimination and extinction contexts, and dopamine dips are used as RPEs only during discrimination. This finding diverges from the RPE mechanism in traditional RL, which may be attributed to the latent state experienced by mice.[418] These results highlight the need for a composite model of biological RL in which animal behaviors generate context-dependent RPEs.

6.5. Other neuromodulatory mechanisms.

6.5.1. Timing-dependent and tonic modulation by GPCRs. According to Regard *et al.*, 308 non-odorant GPCRs are present in the CNS.[419] The mechanisms observed in the striatum are likely to occur in other brain regions as they reflect the general use of AC1 for timing detection. For instance, LTP induced by noradrenaline has been found to result in a 5-second trace in the neocortex, whereas LTD induced by 5HT has a 2.5-second trace,[420] and PKA-induced traces lasting up to 10 minutes have been observed in the hippocampus, and may underlie the long delay conditioning.[421] The plasticity of spines and boutons is correlated to reward conditioning in the prefrontal and orbitofrontal cortex.[422),423] The reward stimuli often trigger glutamatergic input, specifying the consequence of conditioning, as seen in classical conditioning,[381] and place cell formation in the hippocampus by BTSP.[334)–336]

Furthermore, many GPCR agonists tonically regulate the plasticity of spine synapses. For instance, tonic increases in dopamine levels have been linked to psychosis.[424] Neuromodulators, such as acetylcholine (M1R), may act via glial cells to release glutamate, D-serine, and ATP.[425] The Gq-coupled serotonin 5HT2A receptor has been reported to activate Kalirin-7 and facilitate spine morphogenesis.[426] The slow effect of conventional antidepressants may be due to learning processes, while the rapid antidepressant effect of ketamine may be due to the rapid formation of spines (Section 7.5.3).[427]

Although mood is a long-lasting affective state and may have a structural basis (Section 7.5.3), both conditioning and emotion can be short lasting. These phenomena are more closely associated with the acceleration of the enlargement mechanics of existing spines,[398] and direct visualization and quantification of these processes remain challenging.

6.5.2. Drug addiction. Spine synapses play a crucial role in addiction and can arise from exposure to chemical compounds or behaviors that induce dopamine release in the NAc and potentiate causal events, leading to further dopamine release. For example, morphine (via opiate receptors), alcohol, benzodiazepines (via $GABA_A$ receptors), and cannabinoids (via CB receptors) inhibit GABAergic presynaptic terminals of dopamine neurons in the VTA, thereby facilitating dopamine release. In contrast, nicotine, cocaine, and methylamphetamine activate dopamine terminals and tonically increase dopamine concentration. Similar effects were observed for addictive behaviors. One of the consequences of addiction is the enlargement of spine synapses in D1 neurons,[428),429] while another consequence is the weakening of synapses in D2 neurons.[430),431] Many studies have reported changes in spine density and structure in response to addiction.[432),433] Lüscher suggested that withdrawal symptoms are caused by the insertion of calcium-permeable AMPA receptors.[434] Nestler proposed that cocaine addiction is mediated by the generation of small spines and silent synapses, which mature during withdrawal incorporating calcium-permeable AMPA receptors to trigger cocaine-seeking behavior.[435]

7. Intrinsic dynamics and memory management (P4: Instability)

Intrinsic dynamics or fluctuations of synapses represent the quantification of the persistence of spine enlargement and LTP during continual learning. These dynamics have been confirmed by multiple laboratories and account for a significant portion of spine motility over an extended period (Section 7.1). These intrinsic dynamics also serve as homeostatic mechanisms for distributing synaptic weights, facilitating the generation of new spines, and contributing to the slow kinetics of memory decay (Section 7.2). They also require the reactivation of circuits for lifelong persistence (Section 7.3). Furthermore, these dynamics reflect the instability of synapses, and their dysregulation can lead to the spine dysgenesis observed in ASD (Section 7.4).

Notably, intrinsic dynamics should not be regarded as a negative aspect of spine dynamics. In contrast, they serve crucial functions in stabilizing the continual learning of neuronal networks with Hebbian synapses (Section 7.3). Intrinsic dynamics also play a significant role in allocating a substantial portion of the spines to encode new memories (Section 7.2.2). Moreover, they contribute to the prolonged persistence of newly acquired memories in a time-dependent manner (Section 7.2.4). These dynamics may give rise to stochastic algorithms for adaptation and creativity (Section 7.4.4). Finally, intrinsic dynamics play a vital role as a site for neuromodulation (Section 7.5).

7.1. Unveiling our discovery of intrinsic dynamics (fluctuations).

7.1.1. Spine motility. In the 1990s, the motility of filopodia, which serve as a precursor to spines, was reported.[49] Spines emerge from dendrites or spine heads and form synaptic contacts.[49),51),436),437] Matus observed that morphing in spine volumes was caused

by actin dynamics occurring over a timescale of a minute.[136] The morphology exhibited small fluctuations around the average for one hour. Two-photon imaging in the Gan and Svoboda labs revealed that spines can be stable over extended periods of time *in vivo* but also display genesis (generation) and elimination (pruning) at a frequency of 1–4% per week. Turnover, which represents the mean of genesis and pruning, can be used to quantify overall spine genesis and pruning. Basal turnover has been found *in vivo* without exception.[281),282),438)]

7.1.2. Intrinsic dynamics (fluctuations). At this stage, an independent investigation by Yasumatsu in the Kasai lab and Minerbi in the Ziv lab aimed to explore the relationship between spine size (volume) and activity-dependent synaptic plasticity.[39),40)] Both groups applied the activity inhibitors APV or TTX to the medium and discovered that a significant portion of daily fluctuations in spine size and PSD95 amounts were activity-independent. They further observed that slow fluctuations contributed to the standard distributions of spine sizes, which were consistently unimodal, skewed, and heavy-tailed (lognormal-like or quasi-lognormal) (Fig. 4C),[64),439)–442)] indicating that the fluctuations affect all synapses without exception. Importantly, these fluctuations occur slowly and do not contradict the long-term persistence of spine enlargement and LTP, which have been recorded for up to 3 hours. Morphing is a fast, actin-based spontaneous spine motility centered around the mean on a minute scale; however, the fluctuations also include slow changes over days and can give rise to spine pruning and compensatory genesis, representing the dynamics of PSD. Fluctuations are also referred to as intrinsic dynamics, which contrast with the extrinsic dynamics caused by activity-dependent synaptic plasticity.[6),443)] They are also known as synaptic volatility because they contribute to the disappearance of memory traces in the brain,[444)] and because they represent a lack of complete tenacity of synapses.[445)]

This dynamic conclusion has been supported by *in vivo* two-photon imaging[42),43),74),290),446)] and dissociated cultures.[447)] Spine turnovers continue even though TTX and APV are locally superfused to the surface of the visual cortex *in vivo*.[43)] Basal spine turnovers are generally resistant to MK801.[43),281),368)] Similar spontaneous turnovers and fluctuations are reported in normally reared mice with STED microscopy of the hippocampus and neocortex *in vivo*,[448),449)] as well as with two-photon microscopy in macaque monkeys.[47)]

Mutant mice lacking neuronal activity provide further support for the conclusion regarding spine size distribution. Spine size distributions develop similarly when synaptic transmission is abolished in Munc13-1 and 2 double knockout mice[44),450)] and in mice expressing tetanus toxin throughout the entire developmental period.[45)] Normal presynaptic AZ structures do not require activity.[451)] In addition, spine volume distributions are affected only slightly (albeit significantly) in CaMKII-T286A KI mice[290)] and calcineurin knockout mice,[64)] where LTP and LTD, respectively, are severely impaired. Therefore, the size distribution of spine synapses cannot be attributed to extrinsic dynamics.

Recent support for this conclusion was provided by connectomic analysis using ssEM of the neocortex.[46)] Synaptic connections between neighboring cell spines exhibit bimodal distributions, categorized as either S or L, when plotted on a semilogarithmic scale to highlight their lognormal-like distributions.[6)] Remarkably, in multiple synaptic contacts formed between the same axons and dendrites (*i.e.*, double or triple synaptic contacts), the peaks belong to the same size category (either S or L, binary variation). This is consistent with the idea that they are sculpted by the same activity-dependent plasticity, namely extrinsic dynamics.[1),452)] However, note that the spreading of size distributions around the peak S or L, termed analog variation, is uncorrelated, suggesting that they are a result of intrinsic dynamics.

7.1.3. Spine-volume fluctuation mechanics. The primary source of fluctuations is the thermal agitation of molecules and organelles; however, this is not the sole contributor. The gradual exchange of molecular components, organelles, and metabolites plays a crucial role in the proper functioning of spines, encompassing structural maintenance and connectivity with other neurons.[249),453)–456)] These exchanges occur autonomously and at a measured pace, allowing spines to maintain stability over extended periods, while continually refreshing essential constituents. Furthermore, phagocytic glial cells contribute to this process. Remarkably, these fluctuations also manifest correlations with the presynaptic partner, suggesting coordinated dynamics between the pre- and postsynaptic compartments.[457)] Evolution has likely honed these fluctuations to ensure diverse synaptic functionality, facilitating survival amidst climate changes and other environmental variations. Dysregulation of these mechanisms has been linked to ASD classification (7.4), yet they also contribute to the survival of our species.

7.2. Spine volume distributions and forgetting.

7.2.1. Spine volume distribution. In this section, we offer an intuitive explanation of spine volume distributions, given that the underlying mathematical models have been previously published.[6),39),41),440),458)] Fluctuations in spine volume demonstrate a comparable coefficient of variation ($\mathrm{CV} = \sigma / V$) across different initial volumes, indicating that they exhibit smaller fluctuations when they are small in size.[6),39),40),74)] As a result, smaller spines are more likely to remain near the small peak and occasionally shift towards the larger tail. Conversely, when larger spines undergo volume fluctuations, they are more likely to revert to the smaller peak for stabilization (Fig. 4C, blue). Therefore, spine volume distributions exhibit unimodal, skewed, and heavy-tailed (lognormal-like) properties.

When activity-dependent plasticity occurs, smaller spines are enlarged by extrinsic dynamics, causing a significant increase in the population of spines, and the peak of the distribution shifts to a larger value (Fig. 4C, white). Although the notable but limited impact of plasticity hints at a relatively marginal role of extrinsic dynamics in spine motility, they remain vital for circuit operations due to their capacity to systematically alter the circuits. Intrinsic dynamics are random fluctuations that are not considered activity-dependent plasticity such as homeostatic scaling.[33)] Nonetheless, spine enlargement triggered by extrinsic dynamics is gradually normalized by intrinsic dynamics, allowing the distribution to remain constant.[191)] Therefore, intrinsic dynamics can function as a homeostatic mechanism for the distribution of spine volume.

In our analysis, we adopted the assumption that spines have a lower boundary of $0.02\,\mu\mathrm{m}^3$ (Fig. 4A). When spines cross this boundary, they can be pruned[39)] or converted to filopodia.[459)] Frequent spine pruning is counteracted by spine genesis, although the biological mechanisms underlying this compensation remain unknown (Section 7.5). Both genesis and pruning can be induced in an activity-dependent manner (Fig. 4A). Slight discrepancies between genesis and pruning can result in changes in spine density, which has been observed under various conditions.[27),71)]

7.2.2. Small spines in new memories. Small spines are likely to play a critical role in the formation of new memories. As depicted in Fig. 4A, spine generation is linked to new memory acquisition.[56),73),118),282),285),460)] New spines within the same dendrites can be used to reinforce existing learning.[214),367),461)] Notably, small spines are preferential sites for spine enlargement and LTP, and mostly remain in the small category unless they undergo a series of enlargements. Therefore, small spines likely represent recently formed functional synapses that contribute to the encoding of new memories.

7.2.3. Forgetting curves. In 1885, Ebbinghaus identified a distinctive feature of memory: memories that lasted for the first few days tended to be further retained for a longer period.[462)] To test this hypothesis, he memorized numerous nonsense syllables and attempted to recall them after a specific rest period. If memories decay exponentially over time, as $ke^{-\mathrm{at}}$ does, the preservation of old and new memories would be the same. However, he found that memory preservation over 15 days was not exponential but rather logarithmic, $k/(log[\mathrm{t}] + a)$ (known as the Ebbinghaus forgetting curve).[462)] Recent studies have shown that memory decay follows power rules ($kt^{-\mathrm{a}}$) within 15 days.[463)] In either way, memory decay is slower than expected based on an exponential function, implying that longer stored memories are more persistent. However, the exact mechanism underlying this phenomenon remains unknown because many factors are involved in these processes, including memory circuits, reactivation, overwriting, and the inherent decay of synapses.

7.2.4. Population behavior of spines. The intrinsic dynamics of dendritic spines exhibit slow memory kinetics (logarithmic or power rule). If small spines ($0.03\,\mu\mathrm{m}^3$) persist for an extended period, their average sizes must increase statistically (as depicted in Fig. 4A), leading to prolonged life expectancy because of their distance from the elimination boundary.[39)] Consequently, the average sizes of small spines exhibit slow kinetics for up to 30 days, resembling the characteristics of psychological forgetting curves (Fig. 4B).[462),463)] In this manner, intrinsic dynamics can contribute to the preservation of memory in line with psychological forgetting curves. However, after 30 days, the spines begin to decay exponentially (Fig. 4B), as predicted by the elimination. These findings align with observations that memory decay follows a time course slower than an exponential function with memory strengthening upon reactivation. Further studies to explore the persistence of memory in conjunction with fluctuations and the interference of overwriting effects would be useful (Section 5.3.5).[292),464)]

A remarkable ability of intrinsic dynamics is its capacity to predict various phenomena accurately

using a simple stochastic equation (Fig. 4A, B, legend): Intrinsic dynamics can successfully capture the distribution of spine volumes (Fig. 4C), spine pruning and genesis, spine lifetime, and the stability of Hebbian learning (Section 7.3), psychological forgetting curves (Fig. 4B) and memory reactivation for persistence (Fig. 4B, exponential component) (Section 7.3), and spine dysgenesis in patients with ASD (Fig. 4D) (Section 7.4).

7.3. Intrinsic dynamics and memory reactivation. Intrinsic dynamics are one of the reasons that memory reactivation is crucial. The highly recursive circuit structures of the hippocampus are believed to trigger the reactivation of memory circuits,[465)–468)] which is essential for recent memory formation in humans.[7)] LTM also requires reconsolidation involving various parts of the neocortex responsible for the respective memory modality, which necessitates reactivation of the neocortex.[469)] Hippocampal replay connects to a circuit in the cortex.[470),471)] Cortical networks are sufficiently recurrent, and noise can trigger rehearsal,[472),473)] which can overcome memory interference and intrinsic dynamics. These reconsolidation processes are protein synthesis-dependent (P2), and recall can destabilize memory such as when protein synthesis inhibitors are administered.[474)–476)] The plasticity of inhibitory neurons has also been shown to be critical for memory persistence,[477)] although inhibitory synaptic connections are less stable.[478)]

Furthermore, intrinsic dynamics play a crucial role in stabilizing continuous Hebbian learning and preserving the standard synaptic weight distributions. This is the case for both additive and multiplicative plasticity, where increases in synaptic weights are independent and proportional to the original synaptic weights. In the case of additive Hebbian plasticity, feedback resulting from Hebbian learning by spontaneous firing results in the weight distributions which deviate from the standard spine volume distributions.[479)–481)] On the other hand, multiplicative Hebbian plasticity, which involves a lower LTP amplitude than the LTD amplitude for large synapses, can avoid the abnormal distribution issue. However, under continuous spontaneous activity, synaptic strengths converge to a predetermined set point, eliminating the ability to encode new information as memory.[482),483)] These distribution issues can be resolved by experimentally derived intrinsic dynamics that effectively cancel out the continual learning effect through fluctuations and normalize weight distributions, thereby avoiding saturation and preserving LTM through memory reactivation in the presence of spontaneous activity.[443),459)]

7.4. Spine dysgenesis in ASD.

7.4.1. Spine dysgenesis. Spine dysgenesis was first described by Purpura in 1974 when Golgi-impregnated preparations of postmortem brains from individuals with various causes of mental retardation exhibited immature spines with smaller heads and relatively thinner necks. The hallmark of spine dysgenesis is that, although the size distributions of spines are standard, their average sizes are smaller (Fig. 4D). This phenotype has been reported in a vast majority of mice models of ASD examined (>100), including fragile-X mental retardation (FMRP: translation repressor),[76)] Down syndrome (trisomy 21), Rett syndrome (DNA methylation), Angelman syndrome (ubiquitin-ligase), shank1,[56)] and other mouse mutants,[71)] as well as in marmosets with ASD induced by valproic acid.[484)]

7.4.2. Abnormality in intrinsic dynamics. Abnormalities in intrinsic dynamics have often been implicated in ASD in human and mouse studies. Human studies suggest that impairments in ASD are related to constitutively active molecules such as adhesion molecules, scaffolds, translational control, DNA methylation, and ubiquitination. Spine turnover is increased in many mouse models including FMRP-KO,[43),76)] Rett model,[77)] and BTBR mice.[75)] The Okabe laboratory reported enhanced spine turnover in three mutant animals with human genomic mutations (patDp/+, neuroligin-3 R451C point mutation, and BTBR).[485)] It has been reported that this excess turnover is due to intrinsic dynamics in FMRP-KO mice, as demonstrated by local superfusion of APV and TTX *in vivo*.[43)] In addition, fluctuations in amplitude were exaggerated in FMRP-KO mice.[74)] Mutations in phosphatase and tensin homolog have been found in ASD, leading to increased spine density.[486)]

7.4.3. Mechanisms of spine dysgenesis. The relationship between fluctuations and spine dysgenesis (Fig. 4D) can be explained as follows: During learning, there is a predominant enlargement of small spines, leading to a significant increase in the population of larger spines (Fig. 4C), as discussed in Section 7.2.1. However, when fluctuations are exaggerated, the effects of learning on spine volume distributions are constrained by larger fluctuations (Fig. 4D). In other words, the distributions may align more closely with the distribution of the fluctuations (Fig. 4C). As a result, on average, the spines become smaller. This decrease in average spine size provides

an explanation for spine dysgenesis, even though the distribution of fluctuations in individuals with ASD is similar to that in healthy subjects. In reality, these fundamental distributions may naturally shift towards smaller sizes as a consequence of instability, as suggested in FMRP-KO models.[74] The observed spine dysgenesis occurs through the combined effect of these two mechanisms.

7.4.4. How do these spine abnormalities correlate with ASD symptoms? The common behavioral features of ASD include language impairment, communication disorders, social interaction deficits, and repetitive behaviors. They exhibit unusual talents in a specific field of art or science. Although the origins and symptoms of ASD are highly diverse, general trends may still exist. Assuming that instability is exaggerated (P4), although spine plasticity (P2) and mechanics (P5) are intact, the subject's memory functions may be preserved in certain tasks. Recent memory functions may be potentiated if the number of small silent spines increases, facilitating new memory formation, which may explain the unusual learning abilities of talented subjects. Enhanced memory formation has been reported in shank1-KO[56] and Lrfn2 KO mice.[73] Intrinsic dynamics assist stochastic searching for good connections for creativity.[473),487)–489)] The endurance of memory, however, must be impaired, which is typically observed in the acquisition of language and communication skills because they rely on multimodal and intricate details of memory, which is not easy even with machine learning. Sensory hypersensitivity observed in individuals with ASD may share a common root with language development deficits, which could be attributed to a deficit in the long-term tuning of learning in the sensory cortex, resulting in a low signal-to-noise ratio. The striatum is involved in habitual behaviors, and impairment of its synapses may result in repetitive behaviors, as exemplified by shank-3[490),491)] and neuroligin-3 mutants in the dorsal striatum.[492)] Epilepsy is frequently accompanied by ASD and is commonly ascribed to the channelopathy aspect of ASD.[493)] A theoretical study suggested that impairment of fluctuations and excessive stabilization of synapses can cause epileptic activity.[459)]

7.5. Neuromodulation of filopodia and spine genesis.

7.5.1. Generation of filopodia. It is widely believed that spine generation begins with the formation of a filopodium, a thin finger-like protrusion at the leading edge of various cells that is involved in cell adhesion, sensing of the extracellular environment, and cell-to-cell communication. Filopodia are formulated from a positive curvature of the plasma membrane, partly mediated by the activation of the arp2/3 complex by nucleation-promoting factors such as Wiskott-Aldrich syndrome protein.[494)] Filopodia are highly motile, often making transient contact with presynaptic terminals, and have a typical length of 0.5–3 µm and a diameter of 0.5 µm.[24),49),50),495)] They show biochemical differences from spines.[496)] Cdc42, a major RhoGTPase, forms actin bundles in the filopodia. Filopodia are more frequent in young mice (12% of spines in 1 month) but are reduced in adults (1%).[49),50),495)] Filopodia formation can occur spontaneously,[39),460)] but can also be induced by synaptic activity.[201),202),282),285),497)] Spines are generated from filopodia both spontaneously[39),498)] and in an activity-dependent manner.[50),118),499),500)]

It is possible that the activity-dependent formation of filopodia and spines is caused by enlargement of their small heads by extrinsic dynamics. This is because stable spine generation can be induced by the enlargement of the small nascent head in silent synapses, which makes transient contact with presynaptic terminals. This appears as spine generation because time-lapse imaging normally has an interval of at least 1 day, and the moment of filopodia generation or spine enlargement is difficult to image.[282)] Increases in filopodia generation may also be caused by stabilizing actions of extrinsic activity (Fig. 4A). Filopodia move rapidly in a three-dimensional space, and tracking their dynamics is a formidable task.

7.5.2. Steroids regulate spine genesis. An imbalance in steroid hormones contributes to various mental health conditions, such as depression and anxiety, that involve the regulation of spine turnover, as the hormones generally facilitate spine genesis. Sex steroids (testosterone and estrogen) and adrenocorticosteroids (glucocorticoids and mineralocorticoids) exert their effects through receptors primarily located in the nucleus; however, their peripheral types are expressed in the cytosol and plasma membranes of synaptic regions and are major rapid modulators of spine genesis. They can stimulate PKA, MAPK, phosphoinositide 3 kinase, and RhoGTPases for structural regulations.[501)]

Estrogen is produced in the ovary but is also generated from testosterone by aromatase in presynaptic terminals.[502)] Thus, both estrogen and testosterone may act through estrogen receptors (ER).

Estrogen is known to have rapid non-genomic actions to increase spine density, reference memory, and working memory,[502] whereas tamoxifen-sensitive nuclear estrogen-enhanced NMDA receptors increase LTP and spine density.[503] ER rapidly induces a marked increase in spines by activating Rap via activation of the scaffolding proteins affadin and ERK. Interestingly, the generated spines are "silent synapses" lacking AMPA but not NMDA receptors, which are the preferential site for spine enlargement.[500] Castration reduces the density of the number of spines in the amygdala.[504]

Glucocorticoids and cortisol rapidly induce spine generation within 5 hours *in vivo* but more spine elimination after 12 hours, which causes a net reduction in spine density.[505] Consistently, reduced secretion of adrenocorticosteroids from the adrenal cortex inhibit spine turnover, and the addition of cortisol enhances both spine formation and elimination in one day. Chronic glucocorticoids eliminate learning-associated new spines and disrupt previously acquired memory.[506]

7.5.3. Mood disorders and spines. These observations support the hypothalamic-pituitary-adrenal axis hypothesis of depression,[507] which proposes that the impairment of corticosteroid receptors is the major cause of depression, and that abnormalities in dendritic spine plasticity may cause stress-induced anxiety.[508] Reductions in serotonin and noradrenaline levels also contribute to mood disorders.[79),509] In mouse models of depression, exaggerated spine elimination in the prefrontal cortex has been reported, which is rapidly restored by ketamine, potentially underpinning the acute anti-depressant effect of ketamine.[427] Abnormal regulation of neurosteroids (P4), PKA, and BDNF (P2) often results in depression and anxiety.[510] One hypothesis posits that mood, as a lasting affective state, relates to the genesis of spines, which can be observed and utilized for therapy.[511]

7.6. Sleep and spines. Cortical activity remains active during sleep and consolidates memory by relying on spine enlargement.[288] Sleep is critical for the branch-specific formation of spines,[512] which is dependent on the replay of the same activity during motor performance. REM sleep selectively prunes and maintains newly formed spines.[513] Despite possible enlargement of some specific spines and filopodia, spine sizes tend to be reduced overall by sleep,[441),514),515] possibly due to the reduction of spine enlargement and the dominance of spine fluctuations during sleep, similar to the mechanisms observed in ASD (Section 7.4.3). The synaptic homeostasis hypothesis suggests that daily experience is encoded by an increase in synaptic weight, necessitating homeostatic downscaling during sleep to prevent overexcitability.[516] The phosphorylation hypothesis posits that awake-dependent activation of CaMKII, salt-inducible kinase 3, and ERK modifies the phosphorylation status of synaptic proteins to promote sleep.[517),518] Future research is needed that integrates these seemingly different hypotheses regarding sleep.

8. Mechanical transmission of spine synapse (P5: Mechanics)

8.1. Unveiling our discovery of mechanical transmission.

8.1.1. Actions of fine pushing on presynaptic terminals. Spine enlargement, first reported in 2004 (Section 5.1), compresses the bouton by more than 0.1 µm at the active zone. We predicted that pressure could cause enhancement of exocytosis, because Kishimoto *et al.* found profound facilitation of Ca^{2+}-dependent exocytosis caused by pressure in adrenal chromaffin cells in 2006.[519)–522] Since presynaptic effects were initially difficult to verify, we first published the postsynaptic mechanisms in 2004.[128] Takahashi *et al.* measured the assembly of SNAREs using ratiometric FRET in 2010,[523] and Watanabe *et al.* established fluorescence lifetime imaging of presynaptic boutons.[156] In 2014, we discovered a high FRET region within a bouton that was further enhanced by 100 mM sucrose solution.[524] In the following year, we induced an increase in FRET by spine enlargement in a presynaptic terminal and confirmed that the terminal acted as a pressure sensor. However, the direct measurement of physiological functions is challenging. In 2013, the genetically encoded glutamate sensor iGluSnFR was made available,[525] and it was used by Ucar *et al.* to study hippocampal slices. We reported most results except for the effect of spine enlargement on exocytosis in 2018,[526] and a further exocytosis experiment was published in the end of 2021.[37] These experiments consistently showed persistent increases in glutamate release using iGluSnFR in the presynaptic boutons (<30 min), similar to the increase in FRET. We observed that strong pushing inhibited evoked exocytosis, whereas carefully controlled pushing with deformations of less than 0.15 µm facilitated evoked Ca^{2+}-dependent exocytosis, while strong pushing (>0.15 µm) inhibited evoked exocycytosis. Surprisingly, these pushing actions

were independent of the extracellular and intracellular Ca^{2+} levels.

8.1.2. Actions of spine enlargement on presynaptic terminals. Our investigation revealed that spine enlargement specifically influences the presynaptic terminals of synaptic pairs. To demonstrate this, it is necessary to identify synaptic contacts, which can be challenging using light microscopy.[120] This limitation is particularly relevant to one-photon excitation, where precise alignment of multiple light sources is required for synaptic pair identification. Therefore, we utilized two-photon laser scanning microscopy, which exploits the broadening of the excitation spectrum in two-photon excitation, enabling a single laser source to excite three fluorescent probes.[527] Photomultipliers were employed to collect fluorescence emissions, whereas spatial information was obtained from the scanner position. Thus, two-photon excitation imaging enables precise simultaneous multicolor imaging, which cannot be achieved with one-photon imaging. We selectively examined horizontally aligned synapses with substantial overlap, satisfying the criteria for synapse identification based on postsynaptic Ca^{2+} responses to presynaptic stimulation (Extended Fig. 8 of Ref. 37). Consequently, two-photon excitation provides optical identification of synaptic contacts, and our observations indicate that pressure effects occur exclusively within the contact region. The force generated in the spine can be readily transferred to the presynaptic terminal to cause PREST. This transfer of force is facilitated by the tight connection of the synaptic cleft through multiple synaptic adhesion molecules and its thickness of approximately 20 nm,[528),529] which is within a pushing distance of 50–100 nm.

The pushing effect observed in our study was not caused by retrograde chemical messengers. This was evident from the observation that similar pushing effects did not occur when spines enlarged; however, the spine-necks twitched, pulling the presynaptic terminals instead of pushing them.[37]

8.1.3. New mechanosensing mechanism. Presynaptic effects are referred to as PREST effects. This represents a novel mechano-sensing mechanism where the applied pressure on presynaptic boutons enhances the assembly of SNARE proteins and consequently increases the release probability of presynaptic vesicles for a duration of 20–30 minutes. Notably, unlike well-known mechanosensors, such as PIEZO and TRP channels,[530] this gentle pressure application does not cause an increase in the intracellular calcium concentration ($[Ca^{2+}]_i$), signifying that PREST operates independently of Ca^{2+}. Interestingly, PREST is blocked by latrunculin A, an inhibitor of actin polymerization (Fig. 5). This observation suggests that PREST is mediated by the increased preassembly of SNARE proteins, which boost evoked exocytosis, involving t-SNARE in the plasma membrane and v-SNARE in synaptic vesicles, processes that are supported by the actin cytoskeleton of presynaptic boutons. The precise mechanisms, particularly regarding the persistence of this response (20–30 minutes), remain to be fully elucidated.

8.1.4. Force of spine enlargement. Quantifying the force generated by spine enlargement and its effect on PREST induction in presynaptic terminals has been a challenging endeavor. This is primarily because the synaptic surface of the spine is concealed by a presynaptic bouton, which makes the use of an atomic force microscope cantilever infeasible. To address this issue, we employed PREST to measure this force. Calibration was achieved using osmotic pressure generated by a 20 mM sucrose solution, which can easily diffuse into the synaptic cleft and exert pressure on the active zone from the side of the spine. Our observations confirmed the shrinkage of boutons by approximately 40–100 nm in the presence of the sucrose solution. The 20 mM osmotic pressure elicited increases in both the FRET signal and evoked transmission, similar to those caused by spine enlargement,[526] suggesting that the pressure exerted by spine enlargement is equivalent to 20 mOsm, or 0.5 atm, or $0.5\,kg\,f/cm^2$ (where $1\,M\,Osm = 25\,atm$, $1\,atm = 1.033\,kg\,f/cm^2$), and generates a force of 10 nN for an axon-spine interface of $0.2\,\mu m^2$ (with $1\,atm = 101{,}325\,Pa$, $1\,Pa = 1\,N/m^2$). Interestingly, a pressure of $0.5\,kg\,f/cm^2$ is also characteristic of muscle contraction,[531] which may explain why the spine head is abundant in actin, similar to smooth muscle cells. These findings imply that spines are among the most mechanically potent structures in the brain. However, unlike muscles, spines travel a smaller distance (0.1 µm), making the energy consumption by enlargement and PREST minimal. Therefore, it can be proposed that spine synapses are energy-efficient mechanical synapses.

In 1952, Katz initially reported that a hypertonic sucrose solution (170 mM) enhanced miniature EPSPs (mEPSPs) at the neuromuscular junction.[532] Later, in 1996, Stevens utilized sucrose concentrations ranging from 100 mM to 500 mM to deplete the readily releasable pool for evoked EPSPs via mEPSP.[533] Subsequently, in 2014, we made a

significant discovery of increased SNARE-FRET with 100 mM sucrose,[524] prompting us to gradually reduce osmolarity from 100 mM to 20 mM. Through this adjustment, we observed a selective effect on evoked neurotransmission without involvement in spontaneous synaptic transmission when 20 mM sucrose was used (see Fig. 4 in Ref. 37). Notably, uncontrolled pipette pushing only led to the suppression of evoked synaptic transmission, underscoring the importance of staying within the physiological range of pushing (<120 nm).[37] Through this process, mechanical synaptic transmission was discovered.

The revelation of mechanical transmission as the third mode of synaptic transmission, alongside chemical and electrical transmission, was delayed for several reasons. One reason for this was the lack of a known physiological mechanism for pushing the presynaptic terminal other than spine enlargement. Another reason was that the pushing action must be precisely controlled to be effective. Given that spine enlargement and the resultant presynaptic effects reach a certain equilibrium, the law of "action" and "reaction" implies that the same force is applied to the postsynaptic spine by the presynaptic boutons (Fig. 5; Section 5.3.1), establishing mutual mechanically interaction at both sides of synapses.

8.2. How is it related to existing phenomena? Mechanical synaptic transmission is a backpropagation of associative learning toward presynaptic terminals. One hypothesis is that it operates during STP (Fig. 2).[53] STP was originally discovered by McNaughton using electrical stimulation and field EPSP in the 1980s.[534–538] Its characteristics have been extensively investigated by various laboratories, including the Malenka lab.[539,540] These features show a marked similarity to mechanical synaptic transmission (STE+PREST). First, both are induced by activation of postsynaptic NMDA receptors. Second, unlike LTP, the locus of expression (the final functional alteration) is a presynaptic neurotransmitter release that typically lasts for 10–30 min in the STP.[208,541–543] PREST is a presynaptic phenomenon. Thus, STP can be understood as mechanical transmission without retrograde chemical signaling. Third, both are effectively washed out by postsynaptic whole-cell recording, which abolishes cell motility such as spine enlargement (Section 5.2). Fourth, both STP and STE continuously proceeded to LTP and LTE in many studies.[128,153,206,299,543,544] Lastly, STP is relatively resistant to inhibitors,[208,539] which is similar to STE.[128,213,246,247,374]

The similarities observed are intriguing, considering the varied methodologies and preparations employed. Since the 2000s, many studies have focused on LTP (also referred to as LTE), largely sidelining STP (or STE). This shift is partly due to the preference for low-frequency pairing protocols, which result in less prominent STP, allowing for the study of LTP without noticeable STP.[208] Although it presents technical challenges, presynaptic and postsynaptic events can be separately investigated, which provides an opportunity to clarify the precise properties of STP, STE, and PREST in future research. Moreover, the exerted mechanical force can impact presynaptic functions more than a single signaling cascade, resulting in an unpredictable array of influences.[545]

8.3. Short-term and working memory.

8.3.1. Short-term and working memory. Before delving into the involvement of STP in cognitive function, it is essential to introduce STM. Memory can tentatively be divided into STM and LTM. STM can be further divided into the traditional STM, which is the capacity to hold information in an active, readily available state for a short time interval, and "working memory", which temporarily stores information further for processing purposes, such as during thinking, planning, and decision making, and binds ongoing cognitive processes for the integrity of mind.[19,546,547] The persistent activity of a population of neurons maintains STM.[548–553] However, it is important to note that spiking is not always continuous and synapses need to hold memory during the intermittent period (activity-silent working memory).[554]

An ingenious series of simulation experiments was performed to elucidate the involvement of STSP, including paired-pulse facilitation and depression, which occur instantaneously. In previous studies, the model showed some success in accounting for spike activities in moderately complex artificial networks and firing patterns.[8,555–557] STSP rapidly reactivates the already stored LTM and decays in a few seconds.[8] However, working memory is readily transferred to LTM upon recall; thus, STSP alone does not account for this transfer, nor does it provide an associative mechanism to explain working memory processes.

8.3.2. Working memory with STP. An intriguing observation is that STP is reduced with minimal impairment in LTP in GluA1 KO mice.[558–560] Surprisingly, these mice exhibit impaired spatial working memory with intact spatial reference mem-

ory.[561)–563)] Similarly, NR2A blockade selectively impairs STP[559)] and spatial working memory.[564),565)] The reason for the impairment of STP in these KO mice may be attributed to the specific dynamic features of GluA1 and NR2A in the synapses.[164)] Remarkably, GluA1 and NR2A are listed among the 108 loci most susceptible to schizophrenia in genome-wide association studies.[566)] In general, blockade of the NMDA receptor results in working memory impairment,[567)] which is a core cognitive symptom of schizophrenia.

Eugen Bleuler, in 1911, distinguished schizophrenia, meaning "split mind", from dementia praecox. The key concept was the loosening of associations, reflecting disorganized thinking patterns, psychosis, emotional and social disturbances, and lack of self-awareness. He sagely foresaw that schizophrenia results from disruption of associative networks (P2, P3, and P5) within the brain. Schizophrenia is highly inheritable and associated with genes related to synaptic plasticity.[58)] These genes may also be involved in mechanical transmission, as they share the same induction mechanisms. In a neuronal network model, the fast associative plasticity endows a recurrent neural network with attractive properties that allow novel associations in the prefrontal cortex.[568)] Understanding working memory through realistic synaptic dynamics should be able to address the integrity and associative nature of working memory, as well as provide an explanation for the loosening of the association observed in schizophrenia.

8.4. Synaptic associative mechanics and integrity. Synapses play a pivotal role in dynamic information processing, connecting neurons through elongated axons, and exhibiting a diverse array of plasticity (P1–P5). Neurons employ cellular motility, which is a vital mechanism for adapting to environmental changes while maintaining their structural integrity. While glial cells contribute to the regulation of local neuronal functions,[13)] extensive axons and synapses provide the principal connectivity framework for the entire brain. Neurons efficiently facilitate coupling of cognitive subsystems through an abundance of spines and spikes. These structures serve as a vast reservoir of neural modalities and enable the integration of multiple representations within working memory. The coordination of cognitive processes is achieved through global cell assemblies characterized by correlated activities, enabled by transient fortification of associative synaptic contacts.[15)] A key missing link lies in quantitatively connecting synaptic mechanics and spike patterns, which collectively enable global unification. Energy landscapes that incorporate realistic synaptic dynamics and mechanics offer a promising avenue for quantifying distinctive brain states. Furthermore, these dynamics can reveal the physical interactions within neural networks. By providing unique insights into the integrative nature of mental functions, synaptic mechanics (P1–P5) serve as a foundational basis for deciphering the integrity of brain operations.

9. Conclusions

Recent research has provided fascinating insights into the intriguing structural dynamics of synapses and dendritic spines, which are vital for brain function and disorders. This review highlights five key principles that shed light on these dynamics. The first principle (P1: Stability) — spine structure-function relationships are critical for the maintenance of neuronal function, serving as a foundation for stable self-regulation. The second principle (P2: Extrinsic dynamics) — the fast mechanics, spine enlargement, is the basis for associative learning, memory formation, and the establishment of neural representations. The third principle (P3: Neuromodulation) — G-protein-coupled receptors can accelerate spine dynamics by second messengers, linking spine motility to emotional states. The fourth principle (P4: Instability) — intrinsic dynamics of spines act as self-management systems for memory formation, stability, and decay, thereby enabling long-term continual learning and synaptic flexibility for environmental adaptation. The last principle (P5: Mechanics) — the fast mechanics acts on presynaptic terminals, possibly being utilized for working memory and the integration of cognitive functions. Further experimental and theoretical investigations of the principles are crucial for advancing our understanding of the brain function and its disorders. We look forward to exciting new discoveries that will deepen our knowledge of neural processes and potentially lead to novel approaches for the treatment of neurological and psychiatric conditions.

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Profile

Haruo Kasai was born in Hokkaido in 1957 and graduated from the University of Tokyo in 1981. He earned his Ph.D. from the University of Tokyo in 1985, and was an Alexander von Humboldt fellow at the Max-Planck-Institute for Biophysical Chemistry in Göttingen, Germany, from 1988 to 1990. From 1999 to 2005, Kasai was a Professor of Membrane Physiology at the National Institute for Physiological Sciences in Okazaki, and from 2006 to 2022, he was a Professor of Structural Physiology at the University of Tokyo. He is currently a Professor at the International Research Center for Neurointelligence of the University of Tokyo. Kasai has served on the editorial board of the Journal of Physiology (London) and Neural Systems and Circuits. He has been a member of the Science Council of Japan and the advisory board of the Physiological Society of Japan. He was known for his pioneering work on two-photon uncaging of caged-glutamate compounds even before the invention of optogenetics. Glutamate uncaging allows for the optical manipulation of synapses and the discovery of the fundamental features of spine synapses, including the structure-function relationship, enlargement, dopamine modulation, fluctuations, and presynaptic action. Kasai has received several awards for his notable achievements, including the Tsukahara Award, Uehara Prize, Medical Award of Japan Medical Association, Medal with Purple Ribbon, Imperial Prize, and Japan Academy Prize. He is also a fellow at the International Union of Physiological Sciences (IUPS) Academy.