Discrete synaptic states define a major mechanism of synapse plasticity

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Synapses can change their strength in response to afferent activity, a property that might underlie a variety of neural processes such as learning, network synaptic weighting, synapse formation and pruning. Recent work has shown that synapses change their strength by jumping between discrete mechanistic states, rather than by simply moving up and down in a continuum of efficacy. Coincident with this, studies have provided a framework for understanding the potential mechanistic underpinnings of synaptic plastic states. Synaptic plasticity states not only represent a new and fundamental property of CNS synapses, but also can provide a context for understanding outstanding issues in synaptic function, plasticity and development.

The fact that certain excitatory synapses in the brain can change their strength in response to activity has long captured the imaginations of neuroscientists. These changes might underlie such diverse processes as learning and memory, alterations in coding of information in neural networks, and synaptic development and elimination [1]. Synaptic plasticity of the type discussed here has primarily been studied in the excitatory glutamatergic synapses of the brain, particularly of the hippocampus. This review of synaptic properties and plasticity is confined to the widely studied subset of excitatory glutamatergic synapses that are efferent from hippocampal CA3 pyramidal cells to CA3 and CA1 postsynaptic neurons. These synapses have multiple subtypes of glutamate receptors in their postsynaptic membranes, including AMPA receptors, NMDA receptors) and metabotropic glutamate (mGlu) receptors. Generally speaking, AMPA receptor subtypes mediate ion fluxes across the membrane during synaptic transmission at these synapses, whereas NMDA receptors and mGlu receptors are thought primarily to play a role in inducing or modulating plasticity of the AMPA-receptor-mediated transmission [2].

Persistent activity-dependent increases in synaptic transmission are referred to as long-term potentiation (LTP) [3] and decreases in synaptic transmission are termed long-term depression (LTD) [4,5]. Changes in synaptic strength can result from changes in glutamate receptor function [6], increased or decreased glutamate receptor expression in the postsynaptic density (PSD) [2], or changes in transmitter release, as at hippocampal mossy fiber terminals [7]. Blockade of postsynaptic exocytosis or endocytosis [by disrupting activity of N-ethylmaleimide-sensitive membrane fusion protein (NSF) [8] or inhibiting dynamin] prevents expression of LTP [9–11] and LTD, respectively [12–14]. Insertion of AMPA receptors into the synaptic membrane in an activity-dependent manner has been demonstrated using green fluorescent protein (GFP)-tagged AMPA receptor subunits [15,16]. Together with the finding that NMDA-receptor-mediated excitatory postsynaptic currents (EPSCs) do not change with increases in synaptic efficacy [17–19] (but see Refs [20,21]), these data show that potentiation or strengthening of excitatory synapses in the CA1 and CA3 regions of the hippocampus is associated with the specific recruitment and insertion of AMPA receptors from intracellular pools into the postsynaptic membrane [11,15]. Similarly, activity that causes synaptic depression, or a weakening of synaptic strength, is correlated with the endocytosis of these receptors from the postsynaptic membrane [12,22]. Thus, the response of a postsynaptic cell seems to be correlated with the number of AMPA receptors present on the postsynaptic membrane. In most cases, the insertion and removal of AMPA receptors is triggered by Ca²⁺ influx through NMDA receptors. This has led to the assertion that AMPA receptors are responsible for the expression of synaptic plasticity, whereas NMDA receptors are responsible for its control.

Synaptic states: a mechanism of dictating synaptic strength

A key role of synaptic plasticity is to allow the synapse to operate over a large dynamic range. Two possible models could explain the behavior of synapses over this range. In the first, synapses undergo changes in efficacy by adjusting their strength along a continuum, such that the properties of strengthening or weakening occur in a graded fashion with fixed underlying mechanisms (i.e. the ‘continuum model’). In the second, synapses might exist in different discrete states that represent and underlie different levels of efficacy (i.e. the ‘state model’). In an example of the continuum model utilizing AMPA receptor expression as an underlying mechanism, AMPA receptors are inserted or removed from the synaptic membrane and the cellular mechanisms regulating their insertion or removal do not vary across the whole
dynamic range of the synapse strength. Thus, for example, insertion or removal of AMPA receptors into or out of ‘weak’ and ‘strong’ synapses would occur via the same exocytic or endocytic mechanisms with similar ease (Figure 1). In the state model, the underlying properties of the synapse change with alterations in synaptic strength (Figure 2). This would result in synapses existing in different and discrete states with regard to plasticity.

Recent work suggests that the latter model is most likely: synapses undergoing LTP or LTD do so by moving between different discrete electrophysiologically defined states [23]. Previous studies did not reveal all the mechanisms at work in synaptic plasticity because they recorded activity in large populations of synapses, which can average out the diverse behavior and properties of individual synapses. Hence, the diversity of synaptic states was revealed only when the plastic properties of single or small populations of synapses, for example individual pairs of connected neurons [23], were examined. Five synaptic states have been defined: active, potentiated, depressed, silent, and recently silent. The five states are defined as follows:

**Active**

In the active state, synapses display both AMPA-receptor-mediated and NMDA-receptor-mediated responses and are pluripotent with regard to plasticity: they can be either potentiated or depressed by the appropriate synaptic activity protocol. Both LTP and LTD arising from the active state are NMDA-receptor-dependent.

**Potentiated**

When active synapses undergo LTP, they enter the potentiated state. In the CA3 region, plasticity in the potentiated state differs from that in the active state, because depression (depotentiation) depends not on NMDA receptors but on type-1 mGlu receptors, which are prevalent in this brain region. Mechanisms of plasticity in the potentiated state are thought to differ in the CA1 region [24] and in other areas [25,26], where different mGlu receptor subtypes are prevalent [27].

**Depressed**

When active synapses undergo LTD, they enter the depressed state. According to current published data, this state remains ill-defined, and it might differ little from the active state.

**Silent**

Silent synapses are defined as having normal NMDA-receptor-mediated EPSCs but lacking AMPA-receptor-mediated EPSCs [18,19,28–31]. In the literature, this lack of AMPA receptor response is generally attributed to a deficiency of AMPA receptors in the postsynaptic membrane [18,19,30–32] (but see also Ref. [29]). Such synapses are termed silent because they have no synaptic responses at normal postsynaptic membrane potentials, as the NMDA receptor is subject to voltage-dependent Mg$^{2+}$ block [33,34]. Because these synapses have no AMPA responses to begin with, their responses cannot be depressed; however, they can be potentiated by the same types of synaptic activity that potentiate active synapses.

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**Figure 1.** A continuum model to describe synaptic plasticity. In this model, AMPA receptors are continuously cycling into and out of the synaptic membrane: synaptic potentiation [e.g. long-term potentiation (LTP); right] is caused by a relative increase in the rate of AMPA receptor movement into the membrane (thicker green arrow; the relative thicknesses of the red and green arrows indicate the relative rates of exocytosis or endocytosis) compared with the rate of movement out of the membrane (thinner red arrow). Conversely, synaptic depression (left) is caused by a greater rate of AMPA receptor removal (thicker red arrow) than insertion (thinner green arrow). These rate changes will tend towards an equilibrium with more or fewer AMPA receptors in the membrane respectively. Synaptic strengths of intermediate values (middle) reflect more balanced rates for exocytosis and endocytosis of AMPA receptors at the postsynaptic density (PSD). The essential feature of this model is that neither the mechanism nor the ability to undergo synapse strengthening and/or weakening varies with the strength of the synapse. That is, potentiated and depressed synapses differ only in the number of AMPA receptors in the postsynaptic membrane. Of course, this represents an example of only one potential mechanism among many that could underlie the properties of continuous synaptic plasticity.
orms of active rather than potentiated synapses, in the recently silent state. Like active synapses, recently silent synapses have both AMPA-receptor-mediated and NMDA-receptor-mediated responses, but they differ from active synapses in that they cannot undergo synaptic depression (neither LTD nor depotentiation). Recently silent synapses differ from silent synapses because they have AMPA-receptor-mediated responses, and because they undergo a spontaneous transition into the active state ~30 min after unsilencing. After this delay, they recover the ability to undergo synaptic depression. This depression has the properties of active rather than potentiated synapses, in that it is NMDA-receptor-dependent.

Recently silent

Indeed the potentiation of silent synapses leads to the recently silent state. Like active synapses, recently silent synapses have both AMPA-receptor-mediated and NMDA-receptor-mediated responses, but they differ from active synapses in that they cannot undergo synaptic depression (neither LTD nor depotentiation). Recently silent synapses differ from silent synapses because they have AMPA-receptor-mediated responses, and because they undergo a spontaneous transition into the active state ~30 min after unsilencing. After this delay, they recover the ability to undergo synaptic depression. This depression has the properties of active rather than potentiated synapses, in that it is NMDA-receptor-dependent.

Why have discrete plastic states?

Synaptic plasticity that occurs in a state-dependent manner increases the information-carrying capacity of a synapse, in that the potentiation or depression of a synapse has an historical aspect that is absent from a simple continuum model. In a continuum model, information is coded solely in the current strength of the synapse, whereas a state model adds to the coded information the history of the synapse, because the ability of a synapse to undergo, and mechanisms for undergoing, further plasticity are dictated by previous plastic changes at the synapse. For example, synapses potentiated from the active state (to the potentiated state) differ from synapses potentiated from the silent state (to the recently silent state) in that the first can be depressed and the second cannot. Even unsilenced synapses that have transitioned to the active state retain this memory, because their synaptic depression depends on different receptors from that of potentiated synapses. Discrete states preserve synaptic heterogeneity because no single activity protocol can alter all synapses in the same way, thus maintaining the dynamic range of the synaptic population.

Synapses in the silent state were first described several years ago [28] and have been further characterized at CA1 and CA3 synapses in the hippocampus [18,19,29–31] and at thalamocortical synapses [35]. Synapses in this state are crucial to providing circuits with the largest possible dynamic range for increasing synaptic strength. In the recently silent state, synapses are temporarily protected from depression of the AMPA-receptor-mediated response [23]. Because this is the state that silent synapses transition to, synapses in the recently silent state have great potential importance for neural circuitry: they could prevent widespread AMPA receptor downregulation or synapse elimination, thereby protecting any information stored by recently silent synapses. The 30-minute time window of the recently silent state is probably reflected by short-term cellular changes. Transitioning to the active state brings synapses into the most plastic of the states, in that they are the most readily potentiated or depressed in response to incoming neural information. Depression from the potentiated state back to the active state appears to be mGlu-receptor-dependent, whereas depression from the active state to the depressed state is instead NMDA-receptor-dependent [23] (Figure 2). Previous studies have shown that mGlu receptors and NMDA receptors respond preferentially to different synaptic stimulation protocols [36]. This raises the possibility that state transitions could result in state-dependent ‘tuning’ of synapses to listen to different input characteristics. However, because the same synaptic stimulation protocols were employed for
Depression at active and at potentiated synapses, state-dependent plasticity must invoke additional mGlu receptor regulatory mechanisms. Synapses can be depressed and/or silenced from the active state using identical stimulation protocols [23]. Whether these ‘silenced’ synapses possess the same properties as silent synapses remains to be determined, but such silenced or depressed synapses represent a potential prelude to synapse elimination.

Future questions: what molecular changes could define synaptic states?

Clearly, synapses can undergo plastic changes by switching between different states, but what cellular mechanisms underlie these states? Currently, each state is defined physiologically by AMPA receptor retrieval from the membrane, and/or the triggering of this retrieval. It is important to appreciate that AMPA receptor regulation is probably not the sole property defining a given state. Many other known presynaptic or postsynaptic processes could play a role in the definition of plastic states but, for the context of this review, discussion will be restricted to recent developments that could provide a molecular framework for regulating postsynaptic AMPA receptor recycling in a state-dependent fashion.

**Hypothesis 1: AMPA receptor subunit composition as a mechanism of state-dependence**

AMPA receptor insertion into the synapse is differentially regulated by activity, depending on the subunit composition of the receptor. AMPA receptors are generally thought to be heteromeric channels made up of mixtures of GluR1, GluR2, GluR2 and/or GluR4 subunits; the majority of AMPA receptors in the brain are formed by GluR2 subunits in combination with either GluR1 or GluR3 subunits [37]. GluR1-containing and GluR4-containing receptors are thought to constitute a ‘regulated’ pool of receptors that are driven by synaptic activity to the surface of dendritic shafts and into spines [16,17,38]. By contrast, the delivery of GluR2-containing and GluR3-containing receptors to the synapse does not require synaptic stimulation [16]; these receptor subtypes seem to constitute a pool of AMPA receptors that constantly replace existing synaptic AMPA receptors [22]. It will be important to determine which AMPA receptor subunit combinations prevail in different synaptic states, and whether this can define the ability of a synapse to be potentiated or depressed. Because synaptic depression is still evident in GluR2/GluR3 double knockout mice [39], it is likely that mechanisms independent of GluR2 and GluR3 contribute to LTD and the state-dependence of synaptic depression. GluR1 is thought to be sufficient for the expression of synaptic plasticity [39], so this subunit is likely to play an important role in determining the differential state-dependent abilities of AMPA receptors to be removed from postsynaptic membrane (Figure 3).

**Hypothesis 2: AMPA receptor phosphorylation as a mechanism of state-dependence**

Synaptic strength can be increased or decreased by changes in glutamate receptor function and/or localization through post-translational modifications [6,40]. Western blot analysis of large populations of synapses shows that phosphorylation of the Ca2+/calmodulin kinase II (CaMKII) site (Ser831) on GluR1 increases following LTP, whereas dephosphorylation at the protein kinase A (PKA) site (Ser845) occurs following LTD induction to the depressed state, and dephosphorylation of Ser831 occurs following depotentiation [41,42]. In mice expressing mutations at these PKA and CaMKII sites, synaptic depression is lacking [43]. Stabilization of GluR1 during synaptic potentiation by phosphorylation could inhibit internalization of newly inserted AMPA receptors, similar to the recently silent state, whereas their dephosphorylation could mediate the transition to the active state where unfettered internalization of AMPA receptors is allowed.

Concerning other AMPA receptor subunits, biochemical analyses show that PSD-95/discs large/zonula occludens (PDZ)-domain-containing proteins show differential binding to GluR2, depending on the phosphorylation state of the receptor [44–46]. Specifically, phosphorylation at the protein kinase C (PKC) site Ser880 in the GluR2 C-terminal sequence IESVKI disrupts GluR2 binding to a PDZ domain in glutamate receptor interacting protein (GRIP1) but not binding to protein interacting with C kinase (PICK1). In neurons, activation of PKC by phorbol esters increases Ser880 phosphorylation, causing recruitment of PICK1 to synapses and increasing internalization of surface GluR2. These data show that the phosphorylation state of the GluR2 subunit can regulate AMPA receptor internalization and play a stabilizing or destabilizing role in state-dependent synaptic AMPA receptor expression.

**Hypothesis 3: changes in the molecular composition of the PSD as a mechanism of state-dependence**

AMPA receptor insertion, retention and retrieval are intricately controlled by many PDZ domain-containing proteins. PDZ domains are protein–protein interaction sites that bind ligands such as AMPA receptor subunits by their C termini [47,48]. Examples of PSD proteins crucial for AMPA receptor regulation include stargazin [49,50], PICK [51], GRIP [52], and membrane associated guanylate kinases such as PSD-95 [also known as synapse-associated protein (SAP)90] and SAP97 [53]. What role could PSD proteins play in defining synaptic plasticity states? PSD-95 is a key regulator of synaptic AMPA receptor expression, the current defining property of each state. PSD-95 ubiquitination following glutamate receptor activation leads to AMPA receptor internalization [54]; this is consistent with PSD-95 ubiquitination regulating AMPA receptor internalization in discrete synaptic states. PSD proteins are regulated by synaptic activity and plasticity: for example, global changes in synaptic activity lead to changes in PSD protein turnover and ubiquitination [55]. Synaptic protein profiling shows widespread transcriptional changes occurring at the synapse following increased or decreased synaptic activity by chronic bicuculline or tetrodotoxin treatment. Such coordinated regulation of PSD proteins could define distinct synaptic states at a molecular level, resulting in state-dependent differences in synaptic PSD composition.
Many PSD proteins appear to be as dynamic as AMPA receptors in terms of activity-dependent regulation [56,57]. In response to changes in synaptic activity (caused by bath application of tetrodotoxin or glutamate receptor antagonists) synaptic turnover of PSD95 tagged with green fluorescent protein (GFP) is significantly reduced [56]. Synthetic activity can also differentially redistribute PSD proteins: increased synaptic activity (caused by application of high concentrations of extracellular K+ ) induces a reversible, NMDA-receptor-independent synaptic cluster assembly of GFP-Homer 1c (an mGluR1 interacting protein), whereas glutamate stimulation induces cluster disassembly in a manner that depends on NMDA receptors and voltage-dependent Ca2+ channels [57].

Such activity-dependent regulation of these proteins could control the synaptic localization of their glutamate-receptor binding partners in a state-dependent manner. However, because many of these changes were in response to long-term [55,56] or widespread [55–57] changes in activity, it is difficult to relate these changes directly to the state-dependent changes in synaptic strength that occur over the time course of minutes at only a few synapses.

Concluding remarks
That synapses exist in several discrete plasticity states represents a new paradigm for understanding the mechanistic underpinnings of synaptic plasticity, and perhaps also the roles of such plasticity in higher brain functions. Much work remains to be done to define and understand the mechanisms and roles these states play. Emerging data are beginning to elucidate how synaptic plasticity states could arise. Although these states will probably not be specified by a single simple mechanism, it is likely that their underlying mechanisms will arise from differences in the trafficking of different subtypes of AMPA receptors, from regulation of their protein binding partners, and from post-translational modifications of these receptors and partners (e.g. phosphorylation). It will be of primary mechanistic importance to investigate the role of NMDA receptors in the regulation of plasticity states. Recent data show that this receptor, like the AMPA receptor, is subject to activity-dependent regulation [23,36,58]. If NMDA receptor regulation proves to be state-dependent, this would provide a metaplastic aspect [59] to state-dependent synaptic plasticity. Despite all that remains to be determined about the underlying mechanisms, the
finding that synapses exist in different plastic states demonstrates that the information-carrying capacity of a single synapse is greater than previously recognized and fundamentally changes our understanding of the way information is processed in neural circuits.

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