Mini-review

Postsynaptic silent synapses: evidence and mechanisms

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Abstract

In this review I discuss the evidence that some glutamatergic synapses exist that lack surface-expressed postsynaptic AMPA receptors (AMPARs) but contain NMDA receptors opposed to a functional release site. I have summarised the electrophysiological, anatomical and cell biological evidence for such postsynaptically silent synapses, and data that support the idea of rapid AMPAR insertion at silent synapses during long-term potentiation (LTP). I also discuss recent findings suggesting that developmental and activity-dependent alteration in the postsynaptic glutamate receptor composition is a general principle that occurs for other receptor subtypes. This review is not intended to provide a full discussion of possible presynaptic mechanisms for silent synapses; these are covered in the accompanying recent article [Voronin and Cherubini (this issue)].

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1. Electrophysiology of silent synapses

The first experimental evidence for the idea that some glutamatergic synapses exhibit NMDAR-mediated responses in the absence of an AMPAR-mediated component came from a study comparing the trial-to-trial variability of AMPAR- and NMDAR-mediated synaptic transmission at hippocampal CA1 synapses (Kullmann, 1994). Direct observation of NMDAR-mediated synaptic transmission in the absence of an AMPAR-mediated response came soon after (Isaac et al., 1995; Liao et al., 1995). In these latter studies, in which minimal stimulation was used to activate one or a few synapses, two key observations were made. First, the failure rate (proportion of trials in which no synaptic response was detected) was higher at hyperpolarised holding potentials (at which NMDARs are blocked by Mg2+) than at depolarised potentials (when NMDARs are unblocked; Fig. 1A). The second observation was that in a proportion of experiments no synaptic response at all was detected at hyperpolarised potentials but NMDAR-mediated responses were present at depolarised potentials (Fig. 1B). The simple interpretation of these findings was that some glutamatergic synapses lack postsynaptic AMPARs, but contain NMDARs. These synapses were termed ‘silent synapses’ (Liao et al., 1995).

These original silent synapse experiments were designed to exclude the possibility that a presynaptic change during the experiment could explain the result. Thus, throughout the experiment the stimulation frequency was held constant at all times and the only manipulation performed to reveal the presence of silent synapses was to alter the postsynaptic holding potential (with no interruption in the stimulation). In addition, in experiments where all failures were recorded at hyperpolarised potentials, it was demonstrated that the responses at the depolarised potentials were fully blocked by the NMDAR antagonist D-AP5 (Fig. 1B). Furthermore for these experiments, once data had been collected at the depolarised potential the cell was returned to the hyperpolarised potential to check that the stimulation had not changed somehow so that AMPAR-mediated responses were now being evoked (Fig. 1B). In spite of these efforts, two additional possibilities were also addressed. First, that during depolarisation Cs+ (present in the intracellular solution) effluxed through NMDARs from the recorded cell and transiently increased presynaptic release probability; this was excluded by experiments being performed in 0 mM Mg2+ at a constant hyperpol-
Fig. 1. Electrophysiological characterisation of silent synapses. (A) A lower failure rate is observed at depolarised potentials (holding potential of +60 mV; top left) when both NMDARs and AMPARs contribute to synaptic transmission, than at hyperpolarised potentials (−65 mV; top centre) when only AMPARs mediate transmission (averaged EPSCs for both conditions, top right; EPSC amplitude vs. time for entire experiment, bottom left; amplitude histogram for the two holding potentials, bottom right; reprinted from Liao et al. (1995), http://www.nature.com/). (B) Example of an experiment in which no synaptic response was observed at a hyperpolarised potential (−60 mV), but NMDAR-mediated responses were observed at a depolarised potential (+30 mV; EPSC amplitude vs. time for entire experiment, top panel; examples of consecutive EPSCs [upper traces] and averaged EPSCs [lower traces] for each part of the experiment, lower panel; reprinted from Issac et al. (1995), with permission from Elsevier).
arised holding potential (to allow detection of NMDAR-mediated EPSCs), in which failure rate was compared in the presence and absence of the NMDAR antagonist D-AP5 (Liao et al., 1995). Second, that during the depolarisation Ca\(^{2+}\) influx through postsynaptic NMDARs transiently increased release probability in an LTP-like process involving a retrograde signal; this was excluded by including 10 mM BAPTA in the intracellular solution (which chelates intracellular Ca\(^{2+}\)) in a number of experiments (Liao et al., 1995). Thus, even though this possibility has more recently been revisited by other workers (Gasparini et al., 2000), it seems very unlikely that a change in presynaptic function can explain the electrophysiological description of silent synapses.

Subsequent to these initial findings at hippocampal CA1 synapses in rats and guinea pigs, silent synapses have been detected electrophysiologically by a number of other groups and in a variety of other brain areas; e.g. visual cortex (Rumpel et al., 1998), barrel cortex (Isaac et al., 1997), spinal cord (Bardoni et al., 1998; Li and Zhou, 1998) and cerebellum (Losi et al., 2002). Indeed, silent synapses have been demonstrated in the optic tectum of tadpole (Wu et al., 1996). In addition, a number of studies identified a developmental down regulation of the number of silent synapses (Durand et al., 1996; Wu et al., 1996; Liao and Malinow, 1996; Isaac et al., 1997; Hsia et al., 1998; Rumpel et al., 1998; but see Groc et al., 2002), suggesting that this type of glutamatergic input plays an important role in early development. This suggests a two-step mechanism for the development of excitatory circuits: first a non-functional connection is initially formed that does not participate in basal network activity, but a second step is necessary to unsilence the connection requiring appropriate activity (Durand et al., 1996; Wu et al., 1996; Isaac et al., 1997). This presents an attractive model for the activity-dependent development of cortical circuits.

Interest in silent synapses has persisted largely due to a second finding that was reported in the original studies; that silent synapses can undergo NMDAR-dependent LTP characterised by a rapid appearance of AMPAR-mediated responses (Kullmann, 1994; Isaac et al., 1995; Liao et al., 1995; Fig. 2). This finding suggested that LTP at silent synapses is due to the rapid insertion of AMPARs into synapses that initially lacked AMPARs. This purely postsynaptic mechanism was all that was required to explain this form of LTP, and also answered a number of controversies in the literature regarding the site of expression of NMDAR-dependent LTP. It is this feature of silent synapses that has generated the sustained interest, because it provided the first direct evidence that AMPARs could be rapidly inserted into postsynaptic membranes during LTP. This finding has been a major impetus behind the subsequent intense interest in the roles and mechanisms of AMPAR trafficking in synaptic plasticity (Sheng and Lee, 2001; Malinow and Malenka, 2002).

There is now little debate that one can detect NMDAR-mediated responses in the absence of AMPAR-mediated responses at glutamatergic synapses under certain conditions. Furthermore, most accept that the reason for this is that synthaptically released glutamate can access NMDARs while not activating AMPARs on the postsynaptic membrane. The vigorous debate over recent years has been whether this means that some synapses that have a functional release site contain surface-expressed NMDARs but lack surface-expressed AMPARs at their postsynaptic membrane, and what the mechanism is for LTP at silent synapses. This debate centres on the fact that NMDARs have much higher affinity for glutamate than AMPARs (Patneau and Mayer, 1990). Thus it has been proposed that glutamate could ‘spill over’ from neighbouring active synapses onto the postsynaptic receptors at presynaptically inactive synapses at a sufficiently low enough concentration to only activate NMDARs even though AMPARs were also present in the postsynaptic membrane (Asztély et al., 1997; Kullmann and Asztély, 1998). In this scenario LTP is explained by an increase in release probability at these presynaptically silent synapses. The conditions under which the original silent synapse experiments were done were proposed to enhance this spill over since they were performed at room temperature. This is a condition which, amongst other things, reduces high affinity glutamate transport and so has been proposed to enhance spill over (Asztély et al., 1997). Subsequent to this, evidence was provided for an additional alternative mechanism for silent synapses in which glutamate is released slowly and partially from immature presynaptic terminals at sufficiently low enough concentration to only activate the postsynaptic NMDARs at the opposing postsynaptic membrane even though AMPARs are also present (Choi et al., 2000; Renger et al., 2001). In this scenario LTP at silent synapses is due to a rapid change in the presynaptic release machinery to a mature state in which a high concentration of glutamate is rapidly released so that both NMDARs and AMPARs are activated.

Although certain electrophysiological findings have provided evidence against some of these alternative explanations, most notably against spill over (Gomperts et al., 1998; Montgomery et al., 2001), that these mechanisms play at least a partial role in the phenomenon of silent synapses cannot be excluded. However, there is good evidence that glutamatergic synapses can exist in a state where there are no AMPARs surface expressed at the postsynaptic membrane but that these synapses do express NMDARs and AMPARs can be rapidly inserted at such synapses during LTP (see below). Furthermore, recent studies have uncovered molecular mechanisms for
Fig. 2. NMDAR-dependent LTP at silent synapses involves the rapid appearance of AMPAR-mediated responses. No synaptic response was detected at a hyperpolarised holding potential (−60 mV), but, following a pairing protocol (100 stimuli at 1 Hz at a holding potential of 0 mV), AMPAR-mediated EPSCs were observed (EPSC amplitude vs. time for this experiment, top panel; example consecutive EPSCs [upper traces] and averaged EPSCs [lower traces], lower panel; reprinted from Isaac et al. (1995), with permission from Elsevier).

such rapid activity-dependent regulation of AMPAR surface expression.

2. Anatomy of silent synapses

The first anatomical evidence for glutamatergic synapses lacking AMPARs came from a study using an AMPAR subunit specific antibody raised against the extracellular N-terminal region of GluR1 (Molnar et al., 1993). This was used in live cultured hippocampal neurons to detect surface expressed AMPARs and showed that some spines lacked surface-expressed AMPARs even though intracellular AMPARs were detected within the same spine (Richmond et al., 1996; Fig. 3). This finding was reproduced and extended in a number of studies (e.g. Gomperts et al., 1998; Carroll et al., 1999; Liao et al., 1999; Noel et al., 1999; Pickard et al., 2000), which demonstrated that those synapses lacking surface-expressed AMPARs did contain surface-expressed NMDARs. Thus in culture there is an anatomical substrate for the original postsynaptic interpretation of silent synapses.

Immunogold labelling and electron microscopy were necessary to determine if such postsynaptic silent synapses could be detected in native brain tissue. A series of studies provided evidence that AMPAR number varies over a wide range at excitatory synapses onto CA1 pyramidal neurons while NMDAR number is relatively constant (Nusser et al., 1998; Petralia et al., 1999; Takumi et al., 1999; Racca et al., 2000). At a proportion of synapses no AMPARs were detected even though AMPAR immunonegative synapses expressed NMDARs (Fig. 4). Furthermore the number of AMPAR immunonegative synapses was found to be greater early in development (Fig. 4; Nusser et al., 1998; Petralia et al., 1999), consistent with the electrophysiological findings. Electrophysiological estimates for the incidence of silent synapses at hippocampal CA1 synapses in 2 week old animals are ~30% (Durand et al., 1996), 40% (Isaac et al., 1995) and 61% (Liao et al., 1995). Using immunogold the estimated numbers of silent synapses in animals of a similar age are 28% (Nusser et al., 1998) and ~50% (Petralia et al., 1999). Thus the two techniques provide similar estimates for the number of silent synapses providing evidence that the electrophysiological phenomenon of silent synapses is a result of synapses lacking postsynaptic AMPARs. Mossy fibre synapses in the CA3 region did not show any variation in AMPAR number and no AMPAR immunonegative synapses were detected (Nusser et al., 1998; Takumi et al., 1999). This is consistent with the finding that silent synapses at
Fig. 3. Some glutamatergic synapses in cultured hippocampal neurons lack surface expressed AMPARs. Surface-expressed AMPAR distribution revealed by N-terminal GluR1 antibody labelling of a living neuron (top left). Total AMPAR distribution in the same neuron using a C-terminal GluR1 antibody under permeabilising conditions (top right). Co-localisation of the two signals is shown in the bottom panel, open arrows show synapses lacking surface-expressed AMPARs but containing intracellular AMPARs in spines, filled arrows show synapses expressing AMPARs at the surface (scale bars = 5 µm; reprinted from Richmond et al. (1996), with permission from Elsevier).

Mossy fibre autapses are due to non-functional release sites rather than lack of postsynaptic AMPARs (Tong et al., 1996). Therefore in native brain tissue, as well as in culture, there is anatomical evidence in CA1 hippocampus for silent synapses lacking postsynaptic AMPARs. A note of caution is warranted here however. The interpretation of these immunogold studies relies critically on the sensitivity of the antibodies used. Thus not detecting AMPARs at a synapse could be simply due to a lack of sensitivity of the technique rather than a lack of AMPARs. Although this possibility can not be fully excluded, in two studies increasing the sensitivity by using multiple antibodies directed against AMPAR subunits increased the maximum number of AMPARs detected at immunopositive synapses yet immunonegative synapses were still readily detected (Nusser et al., 1998; Petralia et al., 1999). This suggests that a lack of AMPAR labelling indicates that a proportion of synapses do indeed lack postsynaptic AMPARs. Another issue with the immunogold studies is that based on the immunocytochemical data from dissociated culture that there should be a significant number of AMPARs located on intracellular subsynaptic membranes in the plasma membrane at extrasynaptic sites. Indeed one would expect there to be a pool of such receptors to supply a substrate for the rapid insertion of AMPARs at silent synapses during LTP. Although AMPARs are indeed detected at such locations, it remains puzzling that the numbers of such receptors detected are relatively low (Nusser et al., 1998; Racca et al., 2000).

Although these anatomical studies show the existence of postsynaptically silent synapses, they do not address whether LTP at silent synapses involves the rapid insertion of AMPARs as originally suggested. Studies on cultured hippocampal neurons demonstrated that the numbers of silent synapses decreased during development in...
A proportion of CA1 synapses in native hippocampus lack detectable AMPAR expression at the postsynaptic density. (A) Electronmicrographs showing immunogold localisation of AMPARs at CA1 synapses in tissue from 2-day old (left, a–c: ’P2’), 10-day old (centre, d–f: ’P10’) and 5 week old (right, g–i: ’Ad’) rats (in all electronmicrographs ‘p’ indicates the presynaptic terminal and the subunit specific antibody used is indicated on the left). (B) Electronmicrographs showing immunogold localisation of NMDARs at CA1 synapses from 2-day old (left, a–c: ’P2’), 10-day old (centre, d, e: ’P10’) and 5 week old (right, f, g: ’Ad’) rats. (C) Histogram of the number of AMPAR immunogold particles per synapse for 10 day-old (’P10’) and 5 week-old (’5 weeks’) rats showing a reduction in the number of AMPAR immunonegative synapses with age (scale bars are 0.2 µm; reprinted from Petralia et al. (1999), http://www.nature.com/).

culture (Liao et al., 1999; Pickard et al., 2000) and that this developmental reduction was rapidly regulated by activity and required NMDAR activation (Liao et al., 1999). Recently studies have directly demonstrated that new AMPARs rapidly appear at silent synapses during a form of NMDAR-dependent LTP in hippocampal culture (Fitzjohn et al., 2001; Lu et al., 2001; Pickard et al., 2001; Fig. 5) and that this requires membrane fusion (Lu et al., 2001). Therefore, in dissociated culture at least, NMDAR activation can lead to a form of LTP which is due to the rapid insertion of AMPARs at previously silent synapses. Further studies will be necessary to explore whether this form of LTP has all the hallmarks of LTP at CA1 synapses in intact preparations.

Fig. 4. A proportion of CA1 synapses in native hippocampus lack detectable AMPAR expression at the postsynaptic density. (A) Electronmicrographs showing immunogold localisation of AMPARs at CA1 synapses in tissue from 2-day old (left, a–c: ’P2’), 10-day old (centre, d–f: ’P10’) and 5 week old (right, g–i: ’Ad’) rats (in all electronmicrographs ‘p’ indicates the presynaptic terminal and the subunit specific antibody used is indicated on the left). (B) Electronmicrographs showing immunogold localisation of NMDARs at CA1 synapses from 2-day old (left, a–c: ’P2’), 10-day old (centre, d, e: ’P10’) and 5 week old (right, f, g: ’Ad’) rats. (C) Histogram of the number of AMPAR immunogold particles per synapse for 10 day-old (’P10’) and 5 week-old (’5 weeks’) rats showing a reduction in the number of AMPAR immunonegative synapses with age (scale bars are 0.2 µm; reprinted from Petralia et al. (1999), http://www.nature.com/).

Fig. 5. AMPARs are inserted during LTP in hippocampal neurons in culture. Surface-expressed AMPARs on a living cultured hippocampal neuron detected using antibodies directed against the common extracellular loop region of GluR1-4, before (top) and after (middle) induction of NMDAR-dependent LTP in culture. Colocalisation is shown in the bottom panel, red puncta are new AMPARs inserted during LTP (scale bar is 5 µm; reprinted from Pickard et al. (2001), with permission from Elsevier).
3. Trafficking and silent synapses

Since AMPAR number varies greatly at CA1 synapses and AMPARs can be rapidly inserted into the postsynaptic membrane during LTP, there must be molecular mechanisms present in the dendrites of CA1 pyramidal cells that mediate this regulation of AMPAR surface expression.

A number of studies have identified various proteins that interact with the intracellular C-termini of the AMPAR subunits. Of particular interest were the proteins NSF (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998), GRIP (Dong et al., 1997), ABP (Srivastava et al., 1998) and PICK1 (Dev et al., 1999; Xia et al., 1999), which bind to GluR2. These interactions were found to regulate the surface expression of AMPARs in cultured hippocampal neurons (Dong et al., 1997; Lüscher et al., 1999; Noel et al., 1999; Osten et al., 2000; Iwakura et al., 2001; Passafaro et al., 2001; Perez et al., 2001; Braithwaite et al., 2002; Lee et al., 2002). Furthermore, in electrophysiology experiments, acute blockade of these interactions showed that these proteins rapidly (within minutes) regulate AMPAR function at synapses (Nishimune et al., 1998; Song et al., 1998; Lüscher et al., 1999; Lüthi et al., 1999; Daw et al., 2000; Kim et al., 2001; Lee et al., 2002). Indeed at some synapses blocking the NSF-GluR2 interaction resulted in a rapid silencing of previously functional synapses (Lüthi et al., 1999; Noel et al., 1999) corresponding to a complete loss of surface expressed AMPARs at a proportion of synapses (Noel et al., 1999). This mechanism occurred with that for the expression of NMDAR-dependent LTD (Lüscher et al., 1999; Lüthi et al., 1999), also known to be due to a loss of surface expressed AMPARs and complete silencing of some synapses (Carroll et al., 1999; Lüthi et al., 1999). More recently stargazin, the mutated protein in the Stargazer mouse, has also been shown to regulate AMPAR surface expression and function (Chen et al., 2000; Schnell et al., 2002).

This body of work demonstrates that the molecular mechanisms do indeed exist for the rapid regulation of AMPAR surface expression during LTP. Studies using GFP-tagged recombinant AMPAR subunits in cultured hippocampal slices have now provided a direct link between these trafficking/insertion mechanisms and insertion of AMPARs during LTP and have started to define developmental and subunit specific mechanisms for AMPAR insertion (Malinow et al., 2000; Shi et al., 2001). These studies show that GluR1 subunit containing receptors are inserted during NMDAR-dependent LTP in a CaMKII-dependent mechanism requiring a PDZ interaction with the C-terminus of GluR1 (Shi et al., 1999; Hayashi et al., 2000). In heteromeric AMPARs containing GluR1 and GluR2 subunits, the GluR1 C-terminal interactions are thought to take precedence (Shi et al., 2001). The GluR2 subunit C-terminal interactions appear to be important for a constitutive replacement of the newly inserted GluR1-containing receptors by heteromers containing GluR2 and 3 (Shi et al., 2001). Recently this group have demonstrated that AMPARs are inserted into synapses in vivo in the rodent somatosensory (barrel) cortex in response to experience (Takahashi et al., 2003). Thus not only are AMPARs inserted into synapses during LTP in vitro, the same mechanisms operate in neocortex during plasticity in vivo. Most interestingly from the point of view of silent synapses, there is evidence that heteromers containing GluR1 or GluR4, but not GluR2 and 3 heteromers, are directly inserted into silent synapses (Fig. 6; Zhu et al., 2000; Shi et al., 2001). Insertion of both types of receptor complex is activity- and NMDAR-dependent, although so far, it has only been shown that GluR1-containing receptors are rapidly inserted during LTP at silent synapses (Shi et al., 2001). GluR4 expression in hippocampus is strongly developmentally regulated with high levels in the first week when silent synapses are prevalent. Thus, it is thought that AMPARs containing this subunit are preferentially inserted at silent synapses in the first postnatal week, with GluR1-containing receptors being inserted in the second postnatal week. Both these types of receptors are then thought to be replaced by GluR2 and 3 heteromers in a constitutive and activity-independent mechanism (Malinow et al., 2000; Shi et al., 2001).

In summary, there appear to be a number of mechanisms for the rapid regulation of AMPAR surface expression at synapses. Many of these are subunit specific and differentially dependent on activity and NMDAR activation. The challenge now is to determine how these mechanisms interact in the regulation of native heteromeric AMPARs during long-term synaptic plasticity and to determine how the molecules and processes detect activity and transduce this signal into alterations in AMPAR surface expression.

4. Developmental and activity-dependent alterations in postsynaptic glutamate receptor composition

Activity-dependent AMPAR insertion at silent synapses is just one subset of the changes in the glutamate receptor composition that can occur at synapses. Other alterations occur such as changes in the subunit composition. For example, the GluR2 content of native AMPARs is altered during development at hippocampal (Pickard et al., 2000) and neocortical synapses (Kumar et al., 2002). Moreover, a rapid activity-dependent alteration in GluR2 content has been reported at cerebellar synapses (Liu and Cull-Candy, 2000). The number of surface expressed synaptic NMDARs, which until recently was thought to be relatively stable, can also be
Fig. 6. GluR1 and GluR4 subunit containing receptors are inserted into silent synapses. (A) In cultured hippocampal slices, CA1 pyramidal neurons expressing recombinant GluR4-GFP show less silent synapses compared to neighbouring control neurons (example consecutive EPSCs from control [left] and GluR4-GFP expressing [right] neurons at depolarised [+40 mV; top panels] and hyperpolarised [−60 mV; middle panels] potentials; bottom panels: analysis of success rate [left] and number of silent synapses [right] from all cells). (B) In the presence of D-AP5, fully rectifying homomeric GluR4 AMPAR insertion at silent synapses can be detected in GluR4-GFP expressing (right) but not control (left) neurons (upper and middle panels: consecutive EPSCs from example cells; bottom panel summary data for success rate at the two potentials in control and GluR4-GFP expressing neurons; A&B reprinted from Zhu et al. (2000), http://www.nature.com/). (C) Recombinant fully rectifying GluR1 homomers are inserted into silent synapses by CaMKII activity. In neurons expressing GluR1-GFP and constitutively active CaMKII (‘inf-GluR1-GFP-IRES-CaMKII’, middle) but not in neighbouring control neurons (‘uninf’, left), fully rectifying AMPARs are inserted into silent synapses (lower panels: response amplitude vs. stimulus number for example individual experiments; EPSC traces above show consecutive responses at −60 mV [left] and +40 mV [middle], and superimposed averaged traces for both potentials [right]; scale bars 25 pA, 25 ms for left panel; 10 pA, 25 ms for middle panel). Right panel: summary data showing failure rates for control (open bars, ‘uninf’) and GluR1-GFP+CaMKII expressing neurons (filled bars, ‘inf’) at −60 mV and +40 mV. This shows that a lower failure rate is consistently observed at the hyperpolarised potential for GluR1-GFP+CaMKII expressing neurons demonstrating that fully rectifying GluR1 homomers were inserted at silent synapses in these neurons (reprinted from Shi et al. (2001), with permission from Elsevier).

rapidly regulated under certain conditions (Snyder et al., 2001; Tovar and Westbrook, 2002). As well as absolute number, the subunit composition of the surface expressed receptors can also be rapidly altered by activity (Barria and Malinow, 2002). A number of studies have identified the molecular mechanisms for such regulation of NMDARs (Standley et al., 2000; Roche et al., 2001; Scott et al., 2001; Barria and Malinow, 2002; Carroll and Zukin, 2002). In addition to AMPARs and NMDARs, kainate receptor surface expression may also be rapidly regulated. At developing thalamocortical synapses in the rodent barrel cortex, postsynaptic kainate receptors are regulated by development and appear to be rapidly replaced by AMPARs during NMDAR-dependent LTP (Kidd and Isaac, 1999). A recent study has identified a number of proteins that bind a PDZ motif
on the C-termini of the kainate receptor subunits GluR5 and GluR6 (Hirbec et al., 2003). Two of these proteins, PICK1 and GRIP, also bind C-terminal GluR2 (as discussed above) and these interactors rapidly and differentially regulate AMPARs and kainate receptors at the same synapses (Hirbec et al., 2003).

Overall, these studies reveal a much more complex regulation of postsynaptic glutamate receptor surface expression at synapses than was originally envisaged during the early silent synapse studies. The emerging consensus is that multiple postsynaptic mechanisms exist to regulate, not only the total number of surface expressed glutamate receptors, but also their subunit composition. These mechanisms are often subunit specific, can act in a highly co-ordinated manner, are developmentally regulated, and have differential dependencies on synaptic activity and NMDAR activation.

5. Conclusion

A large number of studies using a variety of electrophysiological, anatomical and cell biological techniques have provided compelling evidence that the original electrophysiologically identified phenomenon of silent synapses results in large part from a lack of surface-expressed AMPARs at synapses which contain surface-expressed NMDARs and that are opposed to a functional release site. They also show that LTP at silent synapses is due to the rapid insertion of AMPARs, for which multiple molecular mechanisms have been identified. Although no additional presynaptic mechanism is needed to explain silent synapses, it is impossible to rule out that other mechanisms involving spill over or whispering synapses do not contribute under some conditions. However, due to the large weight of evidence in favour of a purely postsynaptic mechanism, the case for the original postsynaptic interpretation of silent synapses has only strengthened over the intervening years since their original discovery.

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