

Attracted or Repelled? Look Within

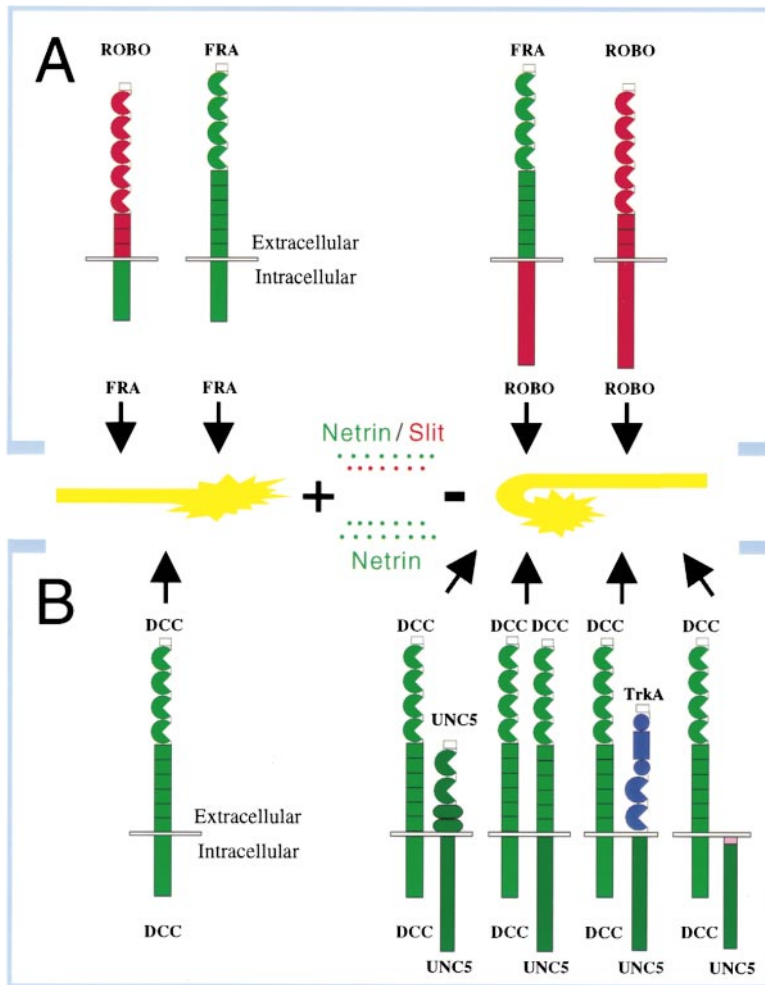
The past several years have seen a revolution in our understanding of how axons find their targets during neural development. These new insights are due in part to a melding of detailed cellular and genetic characterizations of axon guidance events with the molecular description of several families of phylogenetically conserved cues capable of mediating these steering decisions (reviewed by Mueller, 1999). Neurons use these cues, which include both attractants and repellents acting over short and long distances, to direct their axons to appropriate intermediate or final targets. Refinement of these projections is imparted by the ability of individual axons to respond to multiple guidance cues presented at different points along their trajectories. Further complexity in these early guidance events is provided by the bifunctionality of many of these guidance cues: attracting certain populations of axons and repelling others, or even attracting or repelling the same axon depending on the state of certain intracellular signaling molecules. Though it is likely that many families of guidance cues and their receptors remain to be discovered, the tools are now in hand to begin dissecting the molecular basis of attractive and repulsive guidance mechanisms. Two studies published in the June 25 issue of *Cell*, one from the Goodman laboratory (Bashaw and Goodman, 1999) and the other a collaborative effort between the Tessier-Lavigne and Poo laboratories (Hong et al., 1999), provide insight into the logic of how growth cones interpret guidance cues as being attractive or repulsive. Using complementary *in vivo* and cell culture approaches, and focusing on distinct but overlapping guidance receptor families, both groups demonstrate a key role for the cytoplasmic domains of guidance receptors in mediating attraction and repulsion. In addition, Hong et al. (1999) provide evidence for a novel molecular mechanism whereby a heteromultimeric receptor complex can dictate whether the steering response to a single cue, netrin-1 (Net-1), is attractive or repulsive.

Perhaps there is no better place to investigate attractive and repulsive guidance mechanisms than at the CNS midline (Flanagan and Van Vactor, 1998). In both vertebrates and invertebrates, axons from specific populations of neurons are attracted toward the midline by long-range chemoattractants belonging to the Netrin family. Netrin attractive functions are mediated by receptors belonging to the DCC family, a branch of the immunoglobulin (Ig) superfamily that includes DCC in vertebrates and Frazzled (Fra) in *Drosophila*. Upon arriving at the midline, contralaterally projecting axons undergo a conversion; they cross the midline, lose responsiveness to midline-derived Netrin cues, and do not recross the midline. Recent analyses reveal that Slit proteins, also expressed on the midline, can act as repellents and are likely responsible for moving these contralaterally projecting axons across the midline and keeping them from recrossing (Zinn and Sun, 1999). Slit receptors are

members of the Roundabout (Robo) family, also a branch of the Ig superfamily, but they differ from DCC proteins in the number of extracellular Ig and fibronectin domains and share no similarity over their large cytoplasmic domains. Using the well-characterized *Drosophila* midline, Bashaw and Goodman (1999) recognized a great opportunity to test *in vivo* two important and related questions: are Netrin and Slit receptors—Fra and Robo, respectively—modular such that their cytoplasmic domains are responsible for the nature of the growth cone response to a guidance cue; and are attractive and repulsive intracellular signaling components generally present in diverse cell types? The answer to both questions is yes.

Chimeric Fra or Robo receptors, containing either the extracellular domain of Fra and the intracellular domain of Robo (Fra-Robo) or the extracellular domain of Robo and the intracellular domain of Fra (Robo-Fra), were expressed on all neurons in *Drosophila* embryos. This resulted in repulsive guidance responses to Netrins (Fra-Robo) and attractive responses to Slit (Robo-Fra) (see figure, panel A). For example, ectopic Fra-Robo directs axons that would normally cross the midline away from it, leading to a commissureless phenotype. In addition, Fra-Robo also directs motor axons away from the Netrin-expressing muscles that they would normally innervate. These effects are not likely to be due to dominant-negative effects of Fra-Robo. The chimeric Robo-Fra receptor produces complementary phenotypes; Slit can now function as a midline attractant. Remarkably, muscle precursors that normally migrate away from the midline in response to the Slit repellent can interpret Slit as an attractant when they ectopically express Robo-Fra. These results indicate that the attractive or repulsive nature of a particular guidance cue resides in the cytoplasmic domain of the receptor and that this response can be independent of binding a specific class of ligand. Further, a variety of neuronal and nonneuronal cells are shown to be capable of novel attractive and repulsive responses, demonstrating that the downstream signaling components necessary for Netrin and Slit guidance responses are present in different cell types.

But what about guidance cues that are bifunctional—how can the same cue be both an attractant and a repellent? Hong et al. (1999) directly address this issue. Genetic evidence in *C. elegans* and direct evidence in vertebrates have motivated the search for understanding the molecular basis of Netrin bifunctionality (reviewed by Mueller, 1999). Altering intracellular cyclic nucleotide levels in vertebrate neurons *in vitro* can convert an attractive Netrin response to a repulsive one in a DCC-dependent fashion. Though this shows that a single receptor can mediate both Netrin attraction and Netrin repulsion, work in *C. elegans* and in vertebrates demonstrates that UNC5 receptors, yet another branch of the Ig superfamily containing members distinct from both DCC and Robo proteins, are involved in Netrin-mediated repulsive guidance events and are also Netrin binding proteins. However, a simple model whereby DCC and UNC5 receptors independently signal Netrin



The Cytoplasmic Domains of Netrin and Slit Receptors Dictate the Choice between Attraction and Repulsion

attraction and repulsion is challenged by observations that UNC5 and UNC40 (a DCC family member) are both required for many Netrin-mediated repulsive guidance events in *C. elegans*.

First, Hong et al. (1999) directly show, by introducing the vertebrate UNC5H2 receptor into *Xenopus* spinal neurons grown in culture, that an UNC5 receptor is required cell-autonomously to elicit a repulsive response to Net-1. These spinal neurons express endogenous DCC, and previous work showed that antibody neutralization of DCC abolishes an attractive response to Net-1 (Ming et al., 1997). UNC5H2-dependent repulsion is also abolished by DCC neutralization, directly demonstrating a requirement for both UNC5 and DCC for Net-1 repulsion and strongly suggesting that the role of UNC5 is to convert an attractive response to a repulsive one.

To understand how UNC5 effects this conversion, a series of chimeric and altered receptors were constructed and introduced into spinal neurons. Interestingly, Net-1 functions as a repulsive cue in the presence of DCC as long as a membrane-associated cytoplasmic domain of UNC5H2 is also present. A chimeric UNC5H2 consisting of a DCC ectodomain and an UNC5H2 cytoplasmic domain, a similar construct with a TrkA ectodomain, or simply the UNC5H2 cytoplasmic domain targeted to the inner plasma membrane by a myristoylation

sequence are all capable of converting Net-1 attraction to repulsion in the presence of endogenous DCC (see figure, panel B). These data show that Net-1 need not bind the ectodomain of UNC5 to produce repulsion, and they suggest that the cytoplasmic domains of UNC5 and DCC form a receptor complex. This point is shown directly by an extensive series of coimmunoprecipitation experiments which demonstrate that this association is ligand dependent. Therefore, Net-1 binding to its receptor serves to overcome an inhibition of the association between DCC and UNC5 cytoplasmic domains to activate a molecular switch that signals repulsion. Since an UNC5 ectodomain is not required for this switch to occur, Net-1 binding is likely to induce an intramolecular conformational change in DCC that allows the UNC5 and DCC cytoplasmic domains to associate. Additional experiments show that Net-1 can mediate repulsion by binding to the ectodomains of either UNC5 or DCC, so long as both DCC and UNC5 cytoplasmic domains are present. Finally, extensive yeast two-hybrid analysis and in vitro competition experiments define conserved regions of both the DCC and the UNC5 cytoplasmic domains that appear to mediate this association in the absence of additional factors.

Though this work defines certain minimal requirements for converting Netrin attraction to repulsion, it raises

several important questions. What roles do UNC5 ectodomains play in Netrin responses, and do all Netrins within a species interact similarly with DCC and UNC5? Regulation of Netrin-mediated guidance may also result from modulatory events that serve to spatially segregate UNC5 from DCC proteins in those portions of an axon's trajectory where attraction occurs. Future structure-function analyses of DCC and UNC5 extracellular domain associations with DCC, UNC5, Netrin, and possibly other families of proteins will begin to shed light on these issues. In addition, both the Bashaw and Goodman (1999) and Hong et al. (1999) studies raise crucial questions about the nature of repulsive and attractive guidance mechanisms. Might the conversion of the sign of the response to other guidance cues also employ heteromultimeric receptor switches, such that making Semaphorins or Slits attractive is dependent upon the addition or loss of unidentified receptor components? And, finally, though alteration of cyclic nucleotide levels in the growth cone can convert attraction to repulsion and vice versa, does this mean that the signaling outputs for diverse guidance cues and their equally diverse receptors converge on only one or two common signaling pathways? Continued inwardly directed experimental reflection will undoubtedly address these questions.

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Selected Reading

- Bashaw, G.J., and Goodman, C.S. (1999). *Cell* 97, 917-926.
Flanagan, J.G., and Van Vactor, D. (1998). *Cell* 92, 429-432.
Hong, K., Hinck, L., Nishiyama, M., Poo, M.-M., Tessier-Lavigne, M., and Stein, E. (1999). *Cell* 97, 927-941.
Ming, G.-L., Song, H.-J., Berninger, S., Holt, C.E., Tessier-Lavigne, M., and Poo, M.-M. (1997). *Neuron* 19, 1225-1235.
Mueller, B.K. (1999). *Annu. Rev. Neurosci.* 22, 351-388.
Zinn, K., and Sun, Q. (1999). *Cell* 97, 1-4.

The Narp Hypothesis?

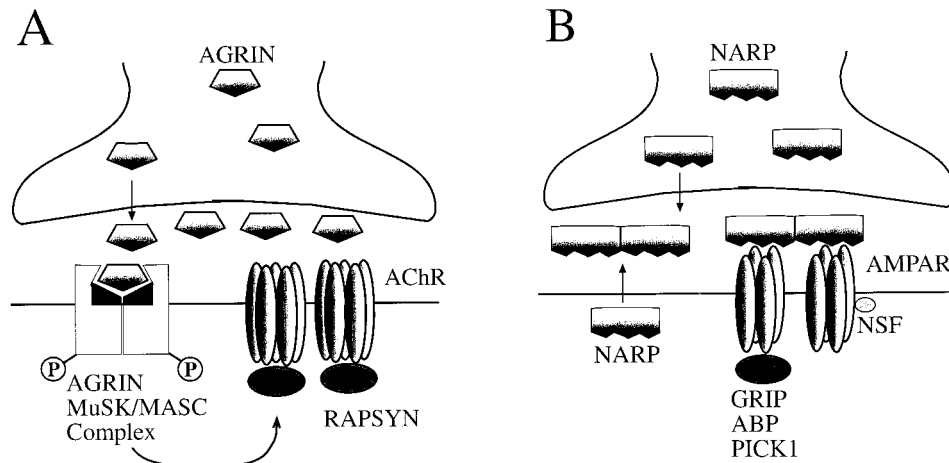
Efficient synaptic transmission requires the enrichment and specific localization of receptors on the postsynaptic membrane apposed to the transmitter release sites. To date, the wealth of information on the vertebrate neuromuscular junction (NMJ) has provided us with the most thorough paradigms of synaptogenesis and synaptic organization (Sanes and Lichtman, 1999). A central player in NMJ formation is agrin, an extracellular heparan sulfate proteoglycan. Agrin is deposited into the synaptic basal lamina by the motor nerve terminal, where it signals transsynaptically through the receptor tyrosine kinase MuSK. Agrin signaling leads to AChR clustering and many other aspects of postsynaptic differentiation. Thus, the agrin hypothesis as proposed by

McMahon (1990) has been well substantiated. Although the exact agrin-induced signaling pathway has yet to be delineated, a key effector protein is rapsyn, a peripheral membrane protein of muscle. Rapsyn can induce clusters of the AChR upon coexpression in heterologous cells and is thought to bind directly to the AChR. Genetic studies in mice have demonstrated the necessary roles of agrin, MuSK, and rapsyn in synaptic differentiation at the NMJ.

Our understanding of mechanisms of receptor clustering at postsynaptic sites on central neurons has been greatly advanced in recent years by identification of CNS receptor binding proteins that may function in an analogous manner to rapsyn. Gephyrin binds to the inhibitory glycine receptor β subunit and is required for postsynaptic clustering of glycine receptors in spinal cord. At glutamatergic synapses, PDZ domain proteins are thought to function in receptor localization and scaffolding to downstream signal transducing proteins (Kim and Haganir, 1999). PDZ domains of the PSD-95 family bind to the C termini (-ESDV) of NMDA receptor NR2 subunits, while PDZ domains of the GRIP family and PICK1 bind to the C termini (-SVKI) of AMPA receptor GluR2/3 subunits. Although direct evidence is lacking for a function of these PDZ domain proteins in localization of NMDA or AMPA receptors at vertebrate glutamatergic synapses, a function in localization of membrane protein ligands and formation of signal transduction complexes has been demonstrated for other PDZ domain proteins in *Drosophila* and *C. elegans*.

In spite of this progress on receptor anchoring/scaffolding proteins of CNS synapses, there has been little progress to date in identifying CNS molecules analogous to agrin, extracellular transsynaptic signaling proteins involved in synaptic differentiation. Agrin itself, though widely expressed in the CNS, is dispensable for the formation of glutamatergic and GABAergic synapses (Serpinskaya et al., 1999). Considering the smaller dimensions of the synaptic cleft at CNS synapses versus at the NMJ, key transsynaptic signaling proteins may be either extracellular or transmembrane. A few transmembrane proteins, notably cadherins, neuroligin, and densin-180, have been localized specifically to CNS synapses, but their function in synaptogenesis has yet to be determined. Enter O'Brien et al. (1999 [this issue of *Neuron*]) with a report of an extracellular protein, Narp (neuronal activity-regulated pentraxin), that can induce clustering of AMPA-type glutamate receptors. Narp was originally cloned by Tsui et al. (1996) as a novel immediate-early gene (IEG) induced by seizure in rat hippocampus. Narp is a member of the pentraxin family of secreted lectins. Classic pentraxins, many of which are acute phase proteins of the immune system, assemble into single symmetric pentameric rings or two such rings interacting face to face.

O'Brien et al. (1999) present several lines of evidence to support a synaptogenic signaling function for Narp at a subset of glutamate synapses. First, Narp is enriched at excitatory synapses on most aspiny but not spiny hippocampal and spinal cord neurons. Second, by analyzing endogenous distribution patterns of surface versus total Narp in hippocampal and spinal cultures, by expressing and localizing myc-tagged Narp in individual



Model for Agrin-Induced AChR Clustering at the Neuromuscular Junction versus Narp-Induced AMPA Receptor Clustering at Some Central Glutamatergic Synapses

spinal neurons, and by preliminary immunogold localization *in vivo*, the authors present strong evidence for both a presynaptic and a postsynaptic source of Narp. For example, expressed myc-Narp could be found coclustered with GluR1 on the dendrites of transfected cells or at contact points between the axons of transfected cells and the dendrites of nontransfected cells. Third, overexpression of myc-Narp in spinal neurons increased the number of postsynaptic GluR1 clusters and increased the number of synaptophysin-labeled presynaptic terminals made onto transfected neurons. Fourth, in heterologous expression studies in HEK cells, Narp formed surface clusters and could induce coclusters of GluR1–3 either when expressed in the same cell or when expressed on apposing cells. Fifth, the association between Narp and AMPA receptor subunits was shown by coimmunoprecipitation from transfected HEK cells and from rat brain. The interaction was not dependent upon N-linked glycosylation and was specific for GluR1–3 and not GluR4, GluR6, or NR1. Finally, and most compellingly, coculture of myc-Narp-expressing HEK cells with spinal neurons induced extrasynaptic clusters of AMPA receptor on the neurons at sites of contact with the myc-Narp clusters. Thus, Narp is an activity-regulated extracellular protein normally present at a subset of glutamatergic synapses that has the ability to induce AMPA receptor clusters.

A hallmark of the significance of this study may be the host of interesting questions it raises for follow-up work. Obviously, the next key experiment will be to perform a functional knockout of Narp to determine whether it is required for AMPA receptor clustering on these neurons or whether it plays a more modulatory role. The possibility that Narp has a more widespread role in synaptogenesis in addition to its effects on AMPA receptor clustering was indicated by the ability of Narp overexpression to increase the number of presynaptic terminals in spinal cultures. Narp also binds taipoxin, a snake venom that blocks synaptic vesicle recycling (Dodds et al., 1997). A modulatory role of Narp in synaptic plasticity is suggested by its restricted distribution to a subset of glutamatergic synapses and its regulation

as an IEG. Remarkably, many of the proteins isolated by Worley and colleagues from a screen for seizure-induced genes in hippocampus have been found to have a primarily synaptic function (Lanahan and Worley, 1998). For example, the IEG product Homer is concentrated at glutamate synapses where it links group I metabotropic glutamate receptors with IP3 receptors (Tu et al., 1998). Narp has now joined the ranks of IEGs that function in synaptic signaling and remodeling.

Additional complexities to be sorted out include cell type expression and selective subcellular targeting of Narp. The current results suggest that Narp may be expressed by both interneurons and some pyramidal cells in the hippocampus but transported selectively to axons of pyramidal cells, but to dendrites of interneurons, and, even more specifically, selectively secreted or stabilized extracellularly at glutamate synapses between pyramidal neurons and interneurons. It is also not entirely clear whether a presynaptic or postsynaptic source of Narp is sufficient for AMPA receptor clustering activity. An attractive model, supported by the effects of myc-Narp and Narp plus GluR1 expression in apposing HEK cells, is that a pre- and postsynaptic source of Narp may be the most effective synaptogenic signal.

Is Narp a functional analog of agrin? There are clear parallels: both are secreted glycoproteins capable of inducing clustering of their respective receptors. However, whereas agrin signals through MuSK and rapsyn to induce aggregation of AChRs, Narp does not require any neuron-specific signaling machinery and indeed may bind directly to AMPA receptors, functioning more like an extracellular rapsyn (see figure). This mechanism may account for the unusual ability of Narp to cluster AMPA receptors in *cis* or *trans*, i.e., whether expressed in the same cell or on apposing cells. Whereas the functionally important splice variants of agrin are derived solely from the presynaptic nerve at the NMJ (Burgess et al., 1999), Narp appears to derive from both pre- and postsynaptic sources. This mechanism leaves in question the roles of NSF and of GRIP1, ABP/GRIP2, and PICK1; perhaps they function in AMPA receptor clustering through an independent pathway or in other

subsets of neurons, or they may have more of a scaffolding function.

A scenario of multiple transsynaptic signaling proteins for different subsets of glutamate synapses is a rather daunting possibility that must now be considered. Such a possibility would allow for the extensive heterogeneity found in molecular composition of individual glutamatergic postsynaptic specializations, depending on pre- and postsynaptic cell type, stage of development, and activity (Rao et al., 1998). Interestingly, there are two proteins closely related to Narp, neuronal pentraxin 1 and neuronal pentraxin receptor (Dodds et al., 1997). The latter has a putative transmembrane domain, and all three can bind to each other in a calcium-regulated manner. These proteins may function in overlapping sets of neurons to regulate glutamatergic synaptogenesis. Considering the complexity and diversity of central synapses, and the specific role of Narp in AMPA receptor clustering, O'Brien et al. (1999) may have opened the first chapter in the "Narp hypothesis" for CNS synaptogenesis.

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Selected Reading

- Burgess, R.W., Nguyen, Q.T., Son, Y.J., Lichtman, J.W., and Sanes, J.R. (1999). *Neuron* 23, 33–44.
- Dodds, D.C., Omeis, I.A., Cushman, S.J., Helms, J.A., and Perin, M.S. (1997). *J. Biol. Chem.* 272, 21488–21494.
- Kim, J.H., and Huganir, R.L. (1999). *Curr. Opin. Cell Biol.* 11, 248–254.
- Lanahan, A., and Worley, P. (1998). *Neurobiol. Learn. Mem.* 70, 37–43.
- McMahon, U.J. (1990). *Cold Spring Harbor Symp. Quant. Biol.* 55, 407–418.
- O'Brien, R.J., Xu, D., Petralia, R.S., Steward, O., Huganir, R.L., and Worley, P. (1999). *Neuron* 23, this issue, 309–323.
- Rao, A., Kim, E., Sheng, M., and Craig, A.M. (1998). *J. Neurosci.* 18, 1217–1229.
- Sanes, J.R., and Lichtman, J.W. (1999). *Annu. Rev. Neurosci.* 22, 389–442.
- Serpinskaya, A.S., Feng, G., Sanes, J.R., and Craig, A.M. (1999). *Dev. Biol.* 205, 65–78.
- Tsui, C.C., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Barnes, C., and Worley, P.F. (1996). *J. Neurosci.* 16, 2463–2478.
- Tu, J.C., Xiao, B., Yuan, J.P., Lanahan, A.A., Leoffert, K., Li, M., Linden, D.J., and Worley, P.F. (1998). *Neuron* 21, 717–726.

Leaky Synapses

The concept that neurotransmitters can act diffusely and at some distance from their release site has long been associated with monoamine- and peptide-mediated synaptic transmission, in which communication is dictated more by the location of the receptors than by the specific site of transmitter release. On the other

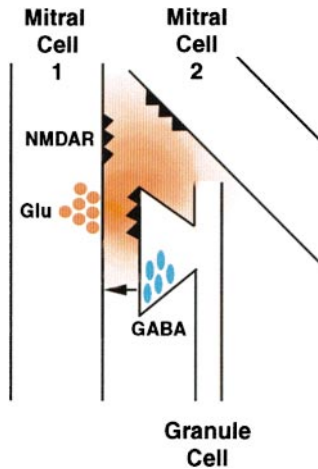


Diagram of Dendrodendritic Inhibition in the Olfactory Bulb and of Glutamate Spillover from One Mitral Cell Dendrite onto a Neighboring Mitral Cell Dendrite

hand, based on the structure and a number of the physiological properties of fast synapses, it has long been assumed that transmission at these synapses is point to point. Yet, during the past few years, evidence has accumulated suggesting that for excitatory synapses glutamate may, under some conditions, be able to spread out of the synapse and activate both pre- and postsynaptic receptors at adjacent synapses. For instance, it has been shown at hippocampal mossy fiber synapses that during repetitive stimulation, glutamate can leave the synaptic cleft and act on presynaptic mGluRs at neighboring synapses (Min et al., 1998; Vogt and Nicoll, 1999). It has also been found that synaptically released glutamate can rapidly activate glutamate transporters on surrounding glial cells (Bergles and Jahr, 1997), indicating that glutamate is capable of spilling out of the synaptic cleft even during low-frequency synaptic activity. Finally, it has been suggested that glutamate spillover may selectively activate postsynaptic NMDA receptors (NMDARs), which are of much higher affinity for glutamate than AMPA receptors; this might explain the occurrence of so-called silent synapses, which exhibit synaptic responses mediated solely by NMDARs (Kullmann and Asztely, 1998). However, the evidence for activation of NMDARs by glutamate spillover has, for the most part, been circumstantial.

In an elegant series of experiments reported in this issue of *Neuron*, Isaacson (1999) has unequivocally shown in the olfactory bulb that the synaptic release of glutamate can spread from one cell and activate NMDARs on a neighboring cell. Mitral cells, the primary relay neurons of the olfactory bulb, release glutamate from their dendrites onto the processes of inhibitory granule cells, which in turn release GABA directly back onto the mitral cell dendrite (see figure) (Jahr and Nicoll, 1982; Isaacson and Strowbridge, 1998; Schoppa et al., 1998). When this inhibitory feedback is removed pharmacologically by a GABA antagonist, a direct self-excitation of mitral cells by glutamate is revealed (Nicoll and Jahr, 1982). Isaacson now shows that this action is entirely due to the direct activation of NMDARs. This synaptic response appears to be very efficient. The release of glutamate

occurs with a probability close to 1, and the open probability of NMDARs when bound by glutamate is high. Finally, the lack of effect of glutamate uptake blockers on the response suggests that glutamate is near saturation.

The ultimate experiment demonstrating the spread of glutamate involves recording from two mitral cells and showing that release of glutamate from one mitral cell can activate NMDARs on the neighboring cell (see figure). Since these responses were recorded after blockade of action potentials with tetrodotoxin and there is no anatomical evidence for direct synaptic interactions between mitral cell dendrites, glutamate must be capable of spreading from one dendrite to another. This response has two features that would be predicted for glutamate acting at a distance. First, the rise time is slow, as expected for a low concentration of glutamate. Second, blockade of glutamate uptake markedly enhances the response, and was often able to bring out a spillover response when one did not exist in control conditions. Finally, evidence is presented suggesting that this spread of glutamate can serve to synchronize the activity of a population of mitral cells and thus contribute to the oscillatory network activity that is presumed to be of importance to the processing of olfactory information. One limitation to this study is that all of the experiments were done in the absence of extracellular Mg^{2+} . This would both enhance glutamate release and allow NMDARs to pass current at hyperpolarized potentials at which, under normal concentrations of extracellular Mg^{2+} , considerably less current would be generated by NMDARs. Thus, in future experiments, it will be important to determine the degree to which spillover of glutamate onto NMDARs plays a functionally important role in synaptic communication within the olfactory bulb.

Recently, the issue of glutamate spillover has received attention because it has been advanced as an alternative explanation for "silent synapses." It is now well established that when one activates only a few excitatory synapses it is possible to record synaptic responses that are mediated entirely by NMDARs with no detectable AMPAR component. Based on this observation, it was postulated that such synapses lacked functional AMPARs (Malenka and Nicoll, 1997). However, if glutamate were able to spill over onto adjacent synapses, the lower concentration might activate the high-affinity NMDARs but fail to activate the lower-affinity AMPARs (Kullmann and Asztely, 1998).

Do the present results have an impact on the silent synapse hypothesis? Probably not. First, the present results were obtained in the olfactory bulb, where glutamate is released from dendrites and acts on extrasynaptic NMDARs. Thus, it is unclear whether one can extrapolate results from this unique synaptic arrangement to other "classical" excitatory synapses. Second, even if spillover of glutamate does occur at other excitatory synapses, this certainly does not exclude the possibility of silent synapses that lack functional AMPARs. Indeed, there is now strong anatomical support for the existence of a population of excitatory synapses which contain NMDARs but not AMPARs (Nusser et al., 1998). In addition, it is possible to record NMDAR-only synaptic responses in autapses, a preparation in which glutamate spillover cannot explain synaptic events mediated only

by NMDARs (Gomperts et al., 1998). Finally, a study of the rise time of the NMDAR response at silent synapses failed to find a slowing as would be expected and, in fact, was found in the present study for the synaptic response generated by glutamate spillover (Haas et al., 1998).

Thus, the present convincing demonstration of spillover of glutamate in the olfactory bulb can live in peaceful coexistence with the silent synapse hypothesis. It is not necessarily an either/or situation. The important question now is whether there is a functional role for spillover of glutamate onto NMDARs at conventional synapses.

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Selected Reading

- Bergles, D.E., and Jahr, C.E. (1997). *Neuron* 19, 1297–1308.
Gomperts, S.N., Rao, A., Craig, A.M., Malenka, R.C., and Nicoll, R.A. (1998). *Neuron* 21, 1443–1451.
Haas, K., Cline, H., and Malinow, R. (1998). *Neuropharmacology* 37, 1393–1398.
Isaacson, J.S., and Strowbridge, B. (1998). *Neuron* 20, 749–761.
Jahr, C.E., and Nicoll, R.A. (1982). *J. Physiol.* 326, 213–234.
Kullmann, D.M., and Asztely, F. (1998). *Trends Neurosci.* 21, 8–14.
Malenka, R.C., and Nicoll, R.A. (1997). *Neuron* 19, 473–476.
Min, M.-Y., Rusakov, D.A., and Kullmann, D.M. (1998). *Neuron* 21, 561–570.
Nicoll, R.A., and Jahr, C.E. (1982). *Nature* 296, 441–444.
Nusser, Z., Lujan, R., Laube, G., Roberts, J.D.B., Molar, E., and Somogyi, P. (1998). *Neuron* 21, 545–559.
Schoppa, N.E., Kinzie, J.M., Sahara, Y., Segerson, T.P., and Westbrook, G.L. (1998). *J. Neurosci.* 18, 6790–6802.
Vogt, K.E., and Nicoll, R.A. (1999). *Proc. Natl. Acad. Sci. USA* 96, 1118–1122.

Thalamocortical Synapses: Sparse but Stentorian

Nearly all of the sensory information that enters the cortex passes through the thalamus, and the most important thalamocortical (TC) projection is onto spiny neurons in layer 4. These TC synapses thus represent the main conduit through which information from the periphery flows into the cortex for further processing. One might imagine that this conduit would be correspondingly wide, but in fact it is remarkably narrow, comprising only about a tenth of all synapses onto a typical neuron in layer 4. The vigorous and rapid responses of

layer 4 neurons to sensory stimulation indicate that TC inputs nevertheless exert a powerful influence, leading to speculation that TC inputs are proportionately more powerful than intracortical (IC) inputs. This prediction was confirmed by Stratford and colleagues (1996), who reported that the TC connection was on average more than twice as strong as its IC counterpart. Now, a study by Gil, Connors, and Amitai (1999 [this issue of *Neuron*]) takes these observations one step further by probing the mechanism underlying this extra strength.

Our basic framework for understanding synapses comes from the classic work of Katz and colleagues (Katz, 1968) on the neuromuscular junction. Katz proposed that when an action potential invades the synaptic terminal, it triggers the release of neurotransmitter from membrane-bound vesicles into the synaptic cleft. Katz developed a simple mathematical model in which he described the size of the postsynaptic response in terms of three quantities: the number (n) of sites from which a vesicle might be released, the probability (p) that a vesicle is released from each site, and the size (q) of the response due to each vesicle. The mean response size, or efficacy (E), is then just the product of these three quantities: $E = q \cdot n \cdot p$.

How do q , n , and p conspire to yield a mean efficacy at TC connections that is more than double that at IC connections? Gil and colleagues conclude that both the number n of release sites and the probability p of release are higher at the TC connection, but that the mean quantal size q is the same. Key to these experiments was the thalamocortical brain slice preparation, in which thalamic and cortical afferents to the same layer 4 neuron can be activated independently. In these special slices, Gil and colleagues first compared the quantal size q in the two pathways by substituting extracellular Sr^{2+} for Ca^{2+} to enhance the asynchronous component of release (Goda and Stevens, 1994). Following a stimulus, this asynchronous component can be seen as a gentle rain of vesicles released by synapses in the stimulated pathway. Because the asynchronous component of release persists at a low rate for a few hundred milliseconds, the currents caused by individual vesicles can be resolved and the size of the individual quanta estimated. The quantal estimates obtained in this way were the same at the TC and IC pathways and were in close agreement with estimates obtained using another approach, in which release probability at individual boutons was reduced to such a low level that, on average, no more than a single vesicle was released.

If the quantal size q is the same, then the increased efficacy of the TC pathway must be due to differences in either the release probability p , the number of release sites n , or both. Even within a single population of synapses, there is a wide distribution of release probabilities. For example, at the well-studied Schaeffer collateral input from hippocampal region CA3 to region CA1, release probability ranges from <0.01 to nearly 1. An estimate of this distribution for the TC and IC pathways was obtained using the NMDA open channel blocker MK-801, which revealed that the TC pathway contained more high-probability synapses than the IC pathway.

Only part of the enhanced efficacy of the TC pathway is due to the increased release probability. To assess the number n of release sites in the two pathways, Gil

and colleagues compared the size of the single-axon evoked response using minimal stimulation. In this technique, the extracellular stimulating current is reduced until only a single fiber is activated. If a connection consists of only a single release site ($n = 1$), as it often does at the hippocampal connection from region CA3 to region CA1, then the quantal size is equal to the single-axon evoked response. If instead the connection consists of more than one release site, as for example at the neuromuscular junction (where n is on the order of a thousand), then the single-axon response may be much greater. This experiment suggested that the average number of release sites at IC connections ($n = 2$) is much smaller than at TC connections ($n = 7$).

These results provide strong evidence for important differences between the TC and IC inputs. However, due to the nature of the preparation, not all desired tests to confirm this can be performed. At the hippocampal CA3–CA1 pathway, where minimal stimulation has become a standard technique, the most rigorous studies apply three independent tests to determine whether stimulation is minimal (Stevens and Wang, 1994; Dobrunz and Stevens, 1997). First, the relationship between response size and stimulus intensity must show a plateau—a region where an increase in stimulus intensity causes no increase in response size, presumably because one fiber is so much closer to the stimulating electrode that there is a broad safety factor before other fibers are recruited. Second, the response must have a fixed shape and latency. Both of these tests were applied in the study by Gil and colleagues. However, at the CA3–CA1 pathway, a third test can be performed that acts as post facto confirmation that in fact only a single synapse contributed to the observed response. Since CA3 Schaeffer collateral axons typically make only a single synapse onto their CA1 target, the average response size when release failures are excluded (the potency) should be independent of release probability. If this condition is met—if, for example, the potency is the same on both pulses during paired-pulse facilitation—then one can be more confident that only a single axon is being stimulated. At cortical synapses, however, this third internal check cannot be performed, since high synapse multiplicity is indistinguishable from supraminimal stimulation. There remains therefore the formal possibility that the apparently high multiplicity really reflects something else—for example, a propensity for cortical axons to travel in bundles and fire in groups. While there is no evidence to support this alternative interpretation, it remains a logical possibility until other techniques can be brought to bear.

The present findings provide a cellular mechanism that helps reconcile the apparently dominant functional role of the TC input in vivo with its relative sparseness. The next step will be to provide a physical interpretation for this increased number of release sites. There are at least three possibilities. First, they could represent distinct boutons made by a single axon, as has been described between pairs of layer 5 neurons (Markram et al., 1997). Second, they could represent multiple active zones at a single bouton. Finally, they could represent multivesicular release at a single active zone. Further work will distinguish these possibilities.

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Selected Reading

Dobrunz, L.E., and Stevens, C.F. (1997). *Neuron* 18, 995–1008.

Gil, Z., Connors, B.W., and Amitai, Y. (1999). *Neuron* 23, this issue, 385–397.

Goda, Y., and Stevens, C.F. (1994). *Proc. Natl. Acad. Sci. USA* 91, 12942–12946.

Stevens, C.F., and Wang, Y. (1994). *Nature* 371, 704–707.

Markram, H., Lubke, J., Frotscher, M., Roth, A., and Sakmann, B. (1997). *J. Physiol.* 500, 409–440.