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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# 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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