11. Materials and methods are available as supporting material on Science Online.
12. Simple linear regression coefficients for each type of phasic response were calculated for each set of data for which probabilities were tested (P = 0.0, 0.25, 0.5, 0.75, and 1.0 in Figs. 2A, B, and E; P = 0.0, 0.25, 0.5, and 0.75 in Fig. 2D). This was done only as an approximation and does not imply linearity in the response functions. In addition to the nonlinear factors discussed in (13), there is imperfection in the subjective timing of the 2-s interval between stimulus onset and potential reward (15). This probably accounts for the small but significant activation of the fully predicted reward in monkey A (Figs. 2C and 3).
13. Unpublished data (30), as well as Figs. 3 and 4, suggest that the responses of dopamine neurons multiply-flight they respond phasically (as shown in Figs. 2A and 3) and could explain why many neurons shown in Fig. 3D appear to have distinct thresholds has critical implica-
tions. Thus, it is not necessarily the case that the maximal responses observed in this study for a given reward magnitude (those at P = 0.0, 0.5, or 1.0) have been on the type of phasic response model as was delayed evoked responses of a given neuron. One would expect that, like other neurons coding the intensity of a signal, dopamine neurons have a stimulus-response function that is insensitive to values above or below a particular range. The likelihood that individual neurons have distinct thresholds has critical implica-
tions for understanding the shape of the probability-
response functions presented in Figs. 2 and 3 and could explain why many neurons shown in Fig. 3D appear to be unresponsive. The shape of the probability functions that we measured would depend on the range of values to which dopamine neurons are sensitive. Because these ranges are unknown, the only interpretation that should be given to the data at this time is that dopa-

14. The present experiments were performed with a standard delay conditioning procedure, meaning that the conditioned stimulus remained on for the full 2-s delay until the potential time of reward. In a separate experi-

15. Objectively, potential reward always occurred after a Z-s delay. However, it is known that subjective timing is variable. The time course of the slowly developing sustained activation could reflect the increasing likelihood that the interval is nearing com-
pletion. Unpublished data (30) on the phasic activa-
tion of dopamine neurons to the delivery of reward earlier or later than predicted suggest a similar de-
gree of temporal imperfection in the prediction. It is therefore reasonable to hypothesize that dopamine neurons may be more uncertain in reward in the sub-
sequent moment (the very near future).
23. The fact that there are two distinct dopamine signals, each with unique properties, suggests two distinct functions for dopamine. However, this does not neces-
sarily imply that the two signals must be processed independently. Thus, each signal may contribute to the performance of two or more functions. Further-

25. In the artificial, impoverished conditions of a laboratory setting or a casino, the probabilities associated with particular stimuli or actions are fixed, and there is nothing else useful to be learned. However, the natural environment contains a high degree of correlation be-
tween multi-sets of events; this is implicit in the adaptive utility of associative learning. Thus, an animal should not assume that uncertainty signals the objec-
tive absence of accurate predictors but rather that it is 

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Identified Sources and Targets of Slow Inhibition in the Neocortex
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There are two types of inhibitory postsynaptic potentials in the cerebral cortex. Fast inhibition is mediated by ionotropic γ-aminobutyric acid type A (GABA_A) receptors, and slow inhibition is due to metabotropic GABA_B receptors. Several neuron classes elicit inhibitory postsynaptic potentials through GABA_A receptors, but possible distinct sources of slow inhibition remain unknown. We identified a class of GABAergic interneurons, the neurogliaform cells, that, in contrast to other GABA-releasing cells, elicited combined GABA_A and GABA_B receptor-mediated responses with single action potentials and that predomin-
antly targeted the dendritic spines of pyramidal neurons. Slow inhibition evoked by a distinct interneuron in spatially restricted postsynaptic compart-
ments could locally and selectively modulate cortical excitability.
GABA$_A$ responses evoked on dendrites (experiments provide evidence for pure regions in the hippocampus (interneurons possibly targeting the dendritic receptor in inhibition is mediated by GABA$_B$ receptors and immunoreactivity for GABA$_B$ receptors, with localization studies indicated a gradient-like (stronger labeling in the upper layers (sensory cortex. Neurogliaform cells (NGFCs, in inhibition in layers 2 to 3 of the rat somato-sensory cortex. Whole-cell recordings with biocytin filling from synthetically coupled pairs of three types of presynaptic interneurons and postsynaptic pyramidal cells, combined with correlated light and electron microscopy, were performed (20, 21). GABA$_A$ receptor localization studies indicated a gradient-like immunoreactivity for GABA$_A$ receptors, with stronger labeling in the upper layers (22). We thus tried to identify the sources of slow inhibition in layers 2 to 3 of the rat somato-sensory cortex. Neurogliaform cells (NGFCs, $n = 78$) were identified on the basis of a late spiking firing pattern and their axonal and dendritic morphology (23–28) (Fig. 1, A and D). Basket cells ($n = 19$) showed a fast spiking firing pattern, received depressing unitary excitatory postsynaptic potentials (EPSPs) arriving from pyramidal cells ($n = 5$), showed immunoreactivity for parvalbumin ($n = 4$ out of 4 tested), and preferentially innervated postsynaptic somata (31%), dendritic shafts (66%) and occasionally spines (3%). Bitufted cells ($n = 15$) responded to depolarizing current pulses with a so-called low-threshold spiking firing pattern (28, 29), received facilitating EPSPs from neighboring pyramidal cells ($n = 3$), placed their synapses onto dendritic shafts and spines (74 and 26%, respectively; $n = 45$), and contained somatostatin ($n = 4$ out of 4 tested). Postsynaptic potentials in pyramidal neurons elicited by NGFCs showed slower ($P < 0.001$, Mann-Whitney test) 10 to 90% rise times (23.4 ± 9.8 ms, $n = 54$) when compared to IPSPs due to basket cell (5.8 ± 2.0 ms, $n = 19$) or bitufted cell (6.5 ± 1.7 ms, $n = 15$) activation (Fig. 1B). The decay of NGFC-to-pyramid IPSPs could not be fitted with single or double exponential functions. We thus measured the half-width of IPSPs for statistical comparison and found that NGFCs to pyramid IPSPs were significantly longer ($P < 0.001$; 183.9 ± 82.5 ms, 61.3 ± 16.3 ms, and 58.9 ± 17.9 ms for NGFC, basket, and bitufted-to-pyramid connections, respectively). Voltage clamp experiments confirmed the conclusions of these recordings (Fig. 1C).

Random electron microscopic sampling of postsynaptic targets showed that NGFCs predominantly innervated dendritic spine necks (30%), spine heads (41%), and dendritic shafts (29%; $n = 65$ target profiles) (Fig. 1D) (27, 28). Three-dimensional light microscopic mapping of NGFC-to-pyramid connections ($n = 8$) confirmed these results and predicted 10 ± 6 synapses on dendritic spines and shafts of pyramidal cells at distances 62 ± 28 µm from the somata. Full electron microscopic analysis of all synapses mapped by light microscopy was performed on a randomly selected pair, and it revealed one synapse on a dendritic spine neck, three on spine heads, and one on a dendritic shaft 63 ± 27 µm (range, 25 to 92 µm) from the soma (Fig. 1, E and F).

NGFC-to-pyramid IPSPs were composed of two components ($n = 21$, Fig. 2, A and B). The early component could be blocked by bicuculline (10 µM, $n = 10$) or gabazine (20 µM, $n = 11$) (Fig. 2C). The early component was blocked by bicuculline (10 µM, $n = 10$) or gabazine (20 µM, $n = 11$) (Fig. 2C).
μM, n = 3), indicating the involvement of GABA<sub>B</sub> receptors (Fig. 2A). Bicuculline or gabazine blockade alone never abolished the response completely and revealed a residual slow component of neurogliaform IPSPs with onset latencies of 60.6 ± 17.3 ms. This late component contributed to the integral of the control IPSPs by 32.1 ± 19.8% and could be blocked by further addition of the GABA<sub>B</sub> receptor antagonist CGP35348 (60 μM). The presence of a postsynaptic GABA<sub>B</sub> receptor–mediated slow component was confirmed by experiments in which the decay of NGFC-to-pyramidal IPSPs was reversibly shortened by CGP35348 (n = 8, Fig. 2B). Although CGP35348 decreased the amplitude of the early component in three out of eight connections, the difference was not significant for the whole data set. The early component was absent at –72 ± 1 mV (n = 8), which was the expected reversal potential for mixed chloride and hydrocarbonate conductance (Fig. 2C); therefore, anion passage through GABA<sub>B</sub> receptors was responsible for the early phase, in agreement with the bicuculline blockade. Hyperpolarization of the postsynaptic cells near the equilibrium potential for potassium ions (–87 ± 2 mV) largely eliminated the late component, consistent with GABA<sub>B</sub> receptor involvement.

The compound IPSPs were highly sensitive to the firing rate of the presynaptic neurons. This could explain why the sources of slow inhibition have remained obscure up to now. We activated the presynaptic NGFCs with single action potentials delivered at various intervals, and a stable amplitude of postsynaptic responses could only be achieved if the interval between presynaptic spikes was more than 1.5 min. Accordingly, all single action potential–evoked responses for the kinetics, pharmacology, and reversal potentials detailed above were collected at especially low presynaptic firing rates (one spike in 100 to 120 s). When the presynaptic NGFCs were activated with trains of action potentials at 40 Hz, the amplitude of postsynaptic responses decreased rapidly (Fig. 2D). Even at a train interval of 4 min (n = 7), postsynaptic responses showed a rapid decrease in amplitude resulting in a complete loss of response after five to eight presynaptic spike trains. After total exhaustion, the recovery of IPSP amplitude was tested with a single presynaptic spike in every 15 min and recovery occurred in all cases. The recovery was initially detectable after 15 to 45 min and reached 31 to 79% of control amplitude as measured 90 min after exhaustion, indicating that the synapses remained functional. The application of high-frequency stimulation or presynaptic interspike intervals above 1.5 min did not have an effect on the kinetics of single spike–evoked events in the same pair.

Our results provide evidence that slow GABA<sub>B</sub> receptor–mediated IPSPs arrive from unitary sources in cortical networks. We identify the first cell type, NGFCs, which consistently recruit postsynaptic GABA<sub>B</sub> receptors in addition to GABA<sub>A</sub> channels. Synapses of NGFCs appear to be specialized for sparse temporal operation tuned for long-lasting metabotropic effects. Although it has been suggested that in some interneuron-to-pyramidal cell connections repeated presynaptic activation might be necessary to recruit slow inhibition (14, 16), single action potentials at very low firing rates are sufficient to elicit the metabotropic GABA<sub>B</sub> component. We cannot rule out, however, the possibility that other type(s) of GABAergic cells might also activate postsynaptic GABA<sub>B</sub> receptors. GABA uptake mechanisms powerfully remove the transmitter from the extracellular space within a distance restricted to about 1 μm from the release sites (30). Our results thus suggest that postsynaptic GABA<sub>B</sub> receptors could be spatially associated with the synapses formed by NGFCs. Electron microscopic studies revealed extrasynaptically placed GABA<sub>B</sub> receptors on dendritic spines and shafts (22, 37–33). A possible synaptic enrichment of these receptors remains to be determined. We showed that the action of NGFCs is predominantly targeted to dendritic spines. The slow rise times of NGFC-to-pyramidal cell IPSPs and inhibitory postsynaptic currents might support the filtering effect of the spine necks. Alternatively, the composition of GABA<sub>A</sub> receptor subunits might influence activation kinetics (34, 35). Although we cannot rule out the possibility that neurogliaform synapses on dendritic shafts and spines act through different receptors, data from the cerebellum suggest that GABA<sub>B</sub> receptors are placed on spines (22, 31). Spines receive the majority of excitatory

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**Fig. 2.** Pharmacology of neurogliaform-to-pyramidal cell connections. Traces show averages ± SEM (gray) of several pairs. (A) The initial component of control IPSPs (n = 10) elicited by single presynaptic action potentials (top) was blocked by bicuculline (10 μM), and the late phase of IPSP was abolished by the subsequent addition of CGP35348 (60 μM). The IPSPs showed recovery after 30 min of washout. (B) The decay of the IPSPs (n = 8) evoked by single spikes in NGFCs (top) could be shortened by application of CGP35348 (60 μM), and this effect could be partially reversed by returning to the control solution. Superimposed traces are shown normalized to the amplitude of control IPSPs (bottom). (C) Voltage–dependent characteristics of the unitary neurogliaform-to-pyramidal IPSPs (n = 6) recorded at membrane potentials of –50, –72, and –87 mV. The early phase shows a reversal potential of –72 mV; the late phase is eliminated at –87 mV. (D) Rapid use–dependent exhaustion of NGFC-to-pyramidal connections demonstrated by a triplet recording with a single presynaptic and two postsynaptic cells. Top, the first five consecutive postsynaptic responses (single sweeps) to presynaptic spike trains elicited at 40 Hz once in 4 min. Bottom, single postsynaptic responses to a presynaptic spike after an inactive period of 30 min show partial recovery.
input, and simulations showed that if inhibitory synapses found on cortical spines are effective, then they should be mediated through GABAB receptors providing powerful hyperpolarizing inhibition that reduces the excitatory synaptic potentials on the same spine (36). In addition to hyperpolarizing inhibitory effects, the diffusion barrier provided by the targeted postsynaptic spines can locally enhance metabotropic changes after GABAB receptor activation. Therefore, even sparse temporal operation of NGFCs could result in sustained modulation of excitability.

References and Notes

21. Materials and methods are available as supporting material on Science Online.
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