When it is on, adipogenesis is repressed; when it is off, adipogenesis is initiated. The crucial role of Wnt signaling in the adipogenic program is emphasized by the finding that in its absence, myoblasts are reprogrammed to the adipocyte lineage and undergo spontaneous differentiation.

References and Notes

CalciuM SensitivitY OF GLUTAMATE RELEASE IN A Calyx-Type Terminal
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Synaptic efficacy critically depends on the presynaptic intracellular calcium concentration ([Ca2+]i). We measured the calcium sensitivity of glutamate release in a rat auditory brainstem synapse by laser photolysis of caged calcium. A rise in [Ca2+]i to 1 micromolar readily evoked release. An increase to >30 micromolar depleted the releasable vesicle pool in <0.5 milliseconds. A comparison with action potential–evoked release suggested that a brief increase of [Ca2+]i to ~10 micromolar would be sufficient to reproduce the physiological release pattern. Thus, the calcium sensitivity of release at this synapse is high, and the distinction between phasic and delayed release is less pronounced than previously thought.

In response to an action potential, the presynaptic release probability is strongly increased for a few milliseconds. This phasic release is thought to be triggered by a brief, localized increase in [Ca2+]i in the vicinity of open, presynaptic Ca2+ channels. The Ca2+ sensitivity of phasic release in mammalian central synapses is not yet known. On the basis of results obtained in other synapses, it has been assumed that a low-affinity Ca2+ sensor, which is activated by local increases of [Ca2+]i to >100 μM, triggers phasic release in mammalian central synapses (1–4). In contrast, the more prolonged, delayed release period that, at most synapses, follows the phasic release may be controlled by a separate Ca2+ sensor with a much higher affinity for Ca2+ (5).

We measured the Ca2+ sensitivity of glutamate release at a giant synapse in the auditory brainstem, the axosomatic synapse formed by the calyx of Held with a principal cell in the medial nucleus of the trapezoid body. Using laser photolysis of caged Ca2+, we compared in the same terminals release evoked by a sustained, spatially uniform rise in presynaptic [Ca2+]i (5), with release triggered by action potentials, during which changes in [Ca2+]i are transient and highly localized (3). In 9-day-old rats, this synapse shows prominent synaptic depression during high-frequency signaling, which is most likely caused by rapid depletion of the releasable pool of vesicles (6–8).

In order to relate the flash-evoked excitatory postsynaptic currents (EPSCs) to the size of the releasable pool in the same terminal, we first established the releasable pool size in the intact terminal. Simultaneous pre- and postsynaptic recordings were made from the calyx and a principal cell (9). With the presynaptic recording still in the cell-associated configuration, a train of action potentials was evoked by an extracellular electrode (Fig. 1A). A measure of release was obtained from the amplitudes of the glutamatergic EPSCs simultaneously recorded in the principal cell. During the train, the size of the EPSCs rapidly depressed, reaching a steady state within 100 ms. The cumulative amplitude of the EPSCs evoked by a train of afferent stimuli (200 ms, 200 Hz) was taken as a measure of the size of the releasable pool (7). This estimate was corrected for the steady-state component in the EPSCs (Fig. 1B). The cumulative EPSC was −9.7 ± 0.7 nA (n = 43, mean ± SEM) at a holding potential of −30 mV. The quantal EPSC amplitude was −32 ± 2 pA (n = 10 cells) at −80 mV. Assuming that the release of one vesicle gives an EPSC amplitude of −12 pA at −30 mV, this gave a releasable pool size of 810 ± 60 vesicles (6, 7). The amplitude of the first EPSC was 21 ± 2% (n = 43) of the amplitude of the cumulative EPSC. Taking the decay of the quantal EPSC into account, this means that about one-quarter of the releasable vesicle pool is released by a single action potential. In the presence of cyclothiazide, the 20 to 80% rise time of a single action potential–evoked EPSC was 424 ± 11 μs (n = 43). Its time course was not different at holding potentials of −80 and −30 mV (paired t test, P > 0.05; n = 7).

After establishing the whole-cell configuration, the terminal was loaded via the patch clamp.

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We fitted the relation between peak release rate and \( [\text{Ca}^{2+}] \), using a kinetic model of the \( \text{Ca}^{2+} \) sensor and its interaction with the releasable vesicles (Fig. 2C). The model features five identical \( \text{Ca}^{2+} \)-binding steps, followed by a final, reversible, \( \text{Ca}^{2+} \)-independent isomerization step that promoted vesicle fusion (22). A satisfactory prediction of the \( [\text{Ca}^{2+}] \), dependence of both the release rates and the delays was obtained with the parameters given in (22). Although this parameter set was not unique, several conclusions could be drawn from the fitting procedure. To reproduce the fast depletion of the pool at high \( [\text{Ca}^{2+}] \), a large isomerization rate constant and fusion rate constant were needed. To reproduce the apparent saturation of release rates at \( [\text{Ca}^{2+}] \) of \( >30 \mu \text{M} \), a dissociation constant \( (K_d) \) of \( \sim 10 \mu \text{M} \) for the individual binding steps was needed, not very different from the estimated affinities of the \( \text{Ca}^{2+} \) sensor that triggers the release of large dense-core vesicles (23–25), but clearly lower than previously estimated for the release of clear vesicles from bipolar cells of the goldfish retina (26).

The laser photolysis experiments can be used to calculate the typical \( [\text{Ca}^{2+}] \), transient observed by a \( \text{Ca}^{2+} \) sensor during action potentials (27). The rise times of the action potential–evoked EPSCs indicated that peak release rates were \( 0.42 \pm 0.04 \text{ ms}^{-1} \) per vesicle \( (n = 43) \). A sustained increase of \( [\text{Ca}^{2+}] \) to \( 5 \mu \text{M} \) gave release rates similar to the ones observed during action potentials (Fig. 3A). This concentration is therefore a lower estimate, because the peak \( [\text{Ca}^{2+}] \) reached during an action potential will be reached only very briefly and will not trigger release as efficiently as a steady increase to the same level.

An upper estimate can be obtained for the \( [\text{Ca}^{2+}] \), transient peak value for the hypothetical situation that all release sites faced the same \( [\text{Ca}^{2+}] \), transient. We assumed that the time course of the \( [\text{Ca}^{2+}] \), transient at the \( \text{Ca}^{2+} \) sensor is not faster than the \( \text{Ca}^{2+} \) current during an action potential (Fig. 3B), which was measured previously (22, 28). With this time course, the amount of release evoked by the simulated \( [\text{Ca}^{2+}] \), transient matched the release evoked by real action potentials if the peak \( [\text{Ca}^{2+}] \), was \( \sim 9 \mu \text{M} \). This estimate was largely model-independent. After adjustment of the parameters of other kinetic models (24, 26, 29) to satisfy the relation between \( [\text{Ca}^{2+}] \), and release rates, a similar estimate was obtained (30). Assuming a linear relation between \( \text{Ca}^{2+} \) influx and the peak of the \( [\text{Ca}^{2+}] \), transient, the simulated action potential–evoked release shared several features with the experimentally characterized release. Delays and rise times of EPSCs were largely independent of the amount of \( \text{Ca}^{2+} \) influx during the action potential (28), although for very high \( \text{Ca}^{2+} \) influx, a decrease in the
synaptic delay and the rise time was observed. The time course of the release probability matched the experimentally observed time course (28). The model predicted a fourth-power dependence of EPSC amplitudes on external [Ca$^{2+}$], somewhat higher than previously measured (6). Our results do not indicate that Ca$^{2+}$ sensors never experience [Ca$^{2+}$], of >10 μM during action potentials. However, they suggest that, in contrast to earlier suggestions (1–4), most Ca$^{2+}$ sensors in the calyx of Held do not experience [Ca$^{2+}$] of hundreds of μM, because even if they were exposed for a brief period, release during action potentials would be faster and larger than experimentally observed.

We conclude that transmitter release from the calyx of Held exhibited a high Ca$^{2+}$ sensitivity compared with previous estimates for the release of clear vesicles from other synapses (18, 26). Our characterization of the Ca$^{2+}$ sensitivity of synaptic transmitter release may be of use in identifying possible Ca$^{2+}$ sensors and in elucidating the molecular mechanisms of transmitter release. For example, synaptogamin I and II are prominent candidates for the Ca$^{2+}$ sensor that triggers phasic release (2, 4). Our results suggest that its binding to synaptxin is unlikely to be involved in the final steps before fusion at the calyx of Held because it requires very high [Ca$^{2+}$]. (2) Synaptic terminals contain a plethora of other Ca$^{2+}$-binding proteins with a higher affinity for Ca$^{2+}$, which may be considered as alternative candidates for the Ca$^{2+}$ sensor (4).

We recorded from calyces of young rats, and the Ca$^{2+}$ sensitivity of release may change during development. However, the observed high sensitivity agrees with other observations. First, [Ca$^{2+}$]$_j$ of <10 μM evoke substantial release in the squid giant synapse (31, 32) and in the crayfish neuromuscular junction (33). Second, the slow Ca$^{2+}$ buffer EGTA not only abolishes delayed release (28, 34, 35), but also affects phasic release at many synapses (28, 34, 36). [Ca$^{2+}$]$_j$ of hundreds of μM are reached only in the immediate vicinity of open Ca$^{2+}$ channels, where EGTA would be ineffective. Third, at the frog neuromuscular junction, EGTA inhibits release, but not the Ca$^{2+}$-dependent potassium channels. This suggests that the Ca$^{2+}$ sensor for release is farther away from the Ca$^{2+}$ channels than the Ca$^{2+}$-dependent potassium channels (37). At the cultured neuromuscular junction, simulations suggest that [Ca$^{2+}$]$_j$ is <10 μM in most regions of a presynaptic Ca$^{2+}$ entry site (38). Combined with our result that a low-affinity Ca$^{2+}$ sensor is not a prerequisite for phasic transmitter release, these results suggest that the high Ca$^{2+}$ sensitivity of phasic release at the calyx of Held may be a property of many synapses.

The phasic-release Ca$^{2+}$ sensor equilibrated rapidly to changes in [Ca$^{2+}$] and triggered release with a high maximal speed, much faster than for dense-core vesicles (39). However, the Ca$^{2+}$ sensitivity observed in the calyx was not very different from the sensitivity of the release of large dense-core vesicles in, for example, melanotrophs (23). Similar Ca$^{2+}$-binding mechanisms may therefore be at work. Finally, because the Ca$^{2+}$ sensor that triggers phasic release has relatively high Ca$^{2+}$ sensitivity, it may be predicted that delayed release is to a large extent a consequence of a delayed triggering of the same sensor, rather than a result of the triggering of a different sensor with much higher affinity.

References and Notes
J. H. Kaplan and G. C. Ellis-Davies, NaCl, 2.5 KCl, 1 MgCl₂, 2 CaCl₂, 25 dextrose, 1.25
10-day-old Wistar rats with a vibratome. The extra-
strate (50
zation. Simultaneous pre- and postsynaptic whole-
lyzed as in (7). It reported a [Ca²⁺]₀ overshoot of
100-μs latency. The time course of [Ca²⁺] was measured
by ≈500% (for 2F², assumed Kᵣ = 0.55
mM⁻¹ s⁻¹) or <50% [for Mag-fura-2, Kᵣ = 0.75
mM⁻¹ s⁻¹ (43)]. Adenosine 5'-triphosphate (ATP)
and endogenous Ca²⁺-buffers were not taken into
account, which should further dampen the initial
[Ca²⁺]₀, spike. The [Ca²⁺]₀ spike is a function of,
for example, the photolysis efficiency (1 to 14% in our
experiments) and [Ca²⁺]₀ buffer conditions (1). In
experiments, release rates observed at similar measured
[Ca²⁺]₀ levels, but with different buffer conditions and
photolysis efficiencies, were similar despite the varying
amplitude of the release wave under these conditions (44). A test of the sensitivity of the Ca²⁺-
sensor (29) to the simulated [Ca²⁺]₀ spikes re-
depend little on the amount of Ca²⁺ release in
each experiment and the presence of CTZ (n = 10 cells).
The time course of the averaged quanta EPSC could be
approximated with a rise time of 130 μs and a
dipexponential decay time constant of 2.5 ms (54%)
and 7.5 ms. Ca²⁺ potentials at millisecond times during an action
were simulated with a Hodgkin-Huxley model (47). Resting [Ca²⁺]₀ in intact terminals was
assumed to be 50 mM (19). It was assumed that the
[Ca²⁺]₀ transients had the same time course as the Ca²⁺ current and were included in all results.
The rationale behind these assumptions is our pre-
vious observation that many Ca²⁺ channels con-
tribute to the release of most vesicles during an action potential at the calyx of Held (28).
This indicates that at most release sites, [Ca²⁺]₀ cannot be expected to change faster than the presynaptic
Ca²⁺ currents.
UV pulses from a frequency-tripled yttrium-aluminium-
ong–Nd laser were coupled into the epifluorescence
port of an Axioskop by means of a quartz light guide
from a quartz light guide garnet–Nd laser were coupled into the epi-
fluorescence port of an Axioskop by means of a quartz light guide
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Uptake of Glutamate into Synaptic Vesicles by an Inorganic Phosphate Transporter

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Previous work has identified two families of proteins that transport classical neurotransmitters into synaptic vesicles, but the protein responsible for vesicular transport of the principal excitatory transmitter glutamate has remained unknown. We demonstrate that a protein that is unrelated to any known neurotransmitter transporters and that was previously suggested to mediate the Na\(^+\)-dependent uptake of inorganic phosphate across the plasma membrane transports glutamate into synaptic vesicles. In addition, we show that this vesicular glutamate transporter, VGLUT1, exhibits a conductance for chloride that is blocked by glutamate.

Synaptic transmission involves the regulated exocytotic release of neurotransmitter. Because most classical transmitters are synthesized in the cytoplasm, they require transport into the secretory compartment for exocytotic release, and synaptic vesicles exhibit multiple distinct transport activities (1, 2). All of these processes depend on the electrochemical gradient (\(\Delta \mu_{\text{H}^+}\)) across the vesicle membrane generated by the vacuolar \(\text{H}^+\)-dependent adenosine triphosphatase (ATPase) (3) and involve the exchange of luminal protons for cytoplasmic transport. In particular, the transport of monoamines and acetylcholine (ACh) depends primarily on the chemical component (\(\Delta \mu_{\text{H}^+}\)) of \(\Delta \mu_{\text{H}^+}\) (4, 5), whereas the transport of glutamate depends predominantly on the electrical component (\(\Delta \psi\)) (6, 7). Accumulation of the inhibitory transmitters \(\gamma\)-aminobutyric acid (GABA) and glycine relies on both \(\Delta \mu_{\text{H}^+}\) and \(\Delta \psi\) (8, 9). Consistent with the observed differences in mechanism, the vesicular transporters for monoamines and ACh belong to a family of proteins distinct from the vesicular GABA transporter (VGAT) (2). VGAT shows greater dependence on \(\Delta \psi\) than do the vesicular monoamine and ACh transporters (10), suggesting that the vesicular glutamate transporter, which depends predominantly on \(\Delta \psi\), might belong to the same family of proteins defined by VGAT. Although several other proteins related to VGAT appear to have a role in the recycling of glutamate through glutamate at excitatory synapses (11–14), none have been implicated in vesicular glutamate transport.

The brain-specific Na\(^+\)-dependent phosphate transporter (BNPI) belongs to a family of proteins that use the inwardly directed Na\(^+\) gradient across the plasma membrane to cotransport inorganic phosphate (P\(_i\)). Originally identified as a sequence upregulated by the exposure of cerebellar granule cells to subtoxic concentrations of N-methyl-D-aspartate, BNPI mediates the Na\(^+\)-dependent accumulation of P\(_i\) in Xenopus oocytes (15). Additional work has implicated BNPI in adenosine 5’-triphosphate (ATP) production by neurons and protection against excitotoxic injury (16, 17). However, BNPI is only expressed by glutamatergic neurons (18), mitigating against a general metabolic role in all neuronal populations. In Caenorhabditis elegans, genetic screens for multiple behavioral defects have identified mutants in the BNPI ortholog eat-4 (19, 20), and recent studies indicate a specific role for eat-4 in glutamatergic neurotransmission (21). The glutamatergic defect in eat-4 mutants appears to be presynaptic, consistent with the localization of BNPI to excitatory nerve terminals (21, 22). The accumulation of cytoplasmic P\(_i\) mediated by BNPI may activate the phosphate-activated glutaminase responsible for biosynthesis of the bulk of glutamate released as a neurotransmitter (22–25). However, the family of proteins including BNPI/EAT-4 may have functions in addition to P\(_i\) transport.

BNPI shows sequence similarity to type I but not type II Na\(^+\)/P\(_i\) cotransporters. In contrast to the type II transporters that exhibit robust Na\(^+\)-dependent P\(_i\) uptake, the accumulation of P\(_i\) by type I transporters is less striking (26–28). Rather, the type I transporter NaPi-1 transports organic anions, including phenol red and penicillin G, with substantially higher apparent affinity than P\(_i\) (28). Human genetic studies have shown that mutations in another protein closely related to BNPI and NaPi-1 account for disorders of sialic acid storage (29). In these conditions, sialic acid accumulates in lysosomes because of a defect in proton-driven export (30–33). Although the sialin protein (29) has not been demonstrated to mediate sialic acid transport, these observations together with the report that NaPi-1 accumulates organic anions with high apparent affinity suggest that BNPI might also transport organic anions. Localization to glutamatergic nerve terminals raises the possibility that it transports glutamate. In addition, BNPI is localized to synaptic vesicles in the brain (22) and to intracellular membranes in transfected cells (34), suggesting a role for BNPI in the transport of glutamate into synaptic vesicles for regulated exocytotic release. To determine whether BNPI mediates the transport of glutamate into synaptic vesicles, we transfected the rat BNPI cDNA into rat pheochromocytoma PC12 cells (35), which lack detectable endogenous BNPI protein (34). We then prepared a population of light membranes, including synaptic-like microvesicles, from the transfected and untransfected cells (10) and tested their ability to accumulate \(^{3}H\)-glutamate in the presence of 4 mM chloride and ATP (36), conditions that optimize glutamate accumulation by native synaptic vesicles (6, 7). Membranes from the transfected cells exhibited an uptake of glutamate that was two to four times the uptake by membranes from

44. Supplemental Web material is available at Science Online at www.sciencemag.org feature/data/1052156.HL.
48. Simulated delays were measured between the [Ca\(^{2+}\)], jump and the time when the integral of the release rate equaled one vesicle. Delays predicted by the model were about 250 \(\mu\)s faster than the measured delays. Ca\(^{2+}\) uncaging is too fast (10 to 20 \(\mu\)s) to contribute substantially to this additional delay (11, 12). Therefore, it probably originates from processes downstream of Ca\(^{2+}\) binding, including vesicle fusion, glutamate diffusion, and activation of AMPA receptors.
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