when inputs are in different modalities^{3,10,11}, are consistent with our own generalized dual-task decrement (Fig. 4). As our results show, it is important that we measured interference time-locked to target presentation. Our experiments deal only with the case of independent visual and auditory inputs; a separate question concerns cross-modal integration when the same event gives rise to input in different modalities¹². Further sources of dual-task interference might also be important in more complex tasks, or when speeded responses are required⁴. Under the simple conditions of our experiments, however, attention to concurrent targets shows no cross-modal restriction. Neurophysiologically, the results suggest that a major source of attentional restriction must lie in modality-specific sensory systems. □

Methods

In each experiment, concurrent input streams were presented at a rate of 250 ms per item. A single target occurred in each stream. In focused-attention conditions, the task was to identify just the target in one specified stream. In divided-attention conditions, both targets were to be identified.

Streams for the single-modality auditory experiment are illustrated in Fig. 1. To create these sequences, single instances of each target and non-target were recorded, then cut to a length of 150 ms and combined into appropriate streams with Macromedia SoundEdit Pro. These streams were presented over headphones at a loudness of approximately 78 dB sound pressure level, with an Apple Macintosh IIci running Cedrus SuperLab software.

Streams for the single-modality visual experiment are illustrated in Fig. 2. Stimuli in this experiment were presented on an Apple Power Macintosh 8500 running Psyscope software¹³. Exact measurements of display timing revealed small variations around the values specified in the program; for example, when stimulus onset asynchrony (SOA) between the two targets was specified as 125 ms, measured values ranged from 119 to 148 ms, and similar levels of variability were seen in target durations and in SOA between one frame and the next within each stream. Such fluctuations were the same under divided- and focused-attention conditions and can accordingly be ignored.

Streams for the mixed-modality experiment are illustrated in Fig. 4. The experiment was run on an Apple Power Macintosh 8500 running Psyscope software, with temporal variability comparable to the visual experiment.

In all experiments, the subject initiated a trial by pressing the space bar of the computer keyboard. Stimulus streams began after a fixed delay of 250 ms; identification responses were typed in after the streams finished, using keys appropriately labelled for the targets 'cot', 'cod', 'nap' and 'nab'. Under focused-attention conditions, there was a single response identifying the attended target (two-alternative forced choice). Under divided-attention conditions, two responses were typed in, in either order. Subjects were strongly encouraged to take time over their responses, ensuring that typing errors were not made. For comparability, the fixation display (central dot and location markers, Fig. 2) was presented in all three experiments, and central fixation was required throughout the trial.

Each subject served in two focused-attention conditions, one for each stream, and the divided-attention condition. Each experiment began with three practice blocks of 32 trials, one per condition. In the auditory experiment there followed a first set of three experimental blocks (96 trials each), one per condition, followed by a second, similar set; other experiments had only one set of three experimental blocks (128 trials each), one per condition. The order of conditions was fixed for any one subject but counterbalanced across subjects. The experiment was conducted in a single session lasting ~1.5 h.

Single-modality auditory and visual experiments had 12 paid subjects each, aged 19–50 years. The mixed-modality experiment had 18 subjects, aged 19–49 years.

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Correspondence and requests for materials should be addressed to J.D. (e-mail: john.duncan@mrc-apu.cam.ac.uk).

The small GTP-binding protein Rab3A regulates a late step in synaptic vesicle fusion

Martin Geppert*, Yukiko Goda†, Charles F. Stevens† & Thomas C. Südhof‡

* Max Planck Institute for Experimental Medicine, Hermann-Rein-Strasse 3, 37075 Göttingen, Germany

† Molecular Neurobiology Laboratory and Howard Hughes Medical Institute, The Salk Institute, La Jolla, California 92037, USA

‡ Department of Molecular Genetics and Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center, Dallas, Texas 75235, USA

The Rab family of low-molecular-mass GTP-binding proteins are thought to guide membrane fusion between a transport vesicle and the target membrane, and to determine the specificity of docking^{1–3}. The docking and fusion of vesicles is, however, a complex multistep reaction, and the precise point at which Rab proteins act in these sequential processes is unknown. In brain, the Rab protein Rab3A is specific to synaptic vesicles, whose exocytosis can be monitored with submillisecond resolution by following synaptic transmission. We have now determined the precise point at which Rab3A acts in the sequence of synaptic vesicle docking and fusion by using electrophysiological analysis of neurotransmitter release in Rab3A-deficient mice. Unexpectedly, the size of the readily releasable pool of vesicles is normal, whereas Ca²⁺-triggered fusion is altered in the absence of Rab3A in that a more-than-usual number of exocytic events occur within a brief time after arrival of the nerve impulse.

We first investigated whether the *rab3A* deletion modifies the properties of the fundamental unit of transmitter release: the quantity of transmitter contained in each vesicle and the basal rate of spontaneous vesicle fusion. We used spontaneous miniature excitatory postsynaptic currents (mEPSCs), each one of which corresponds to exocytosis by an individual vesicle⁴ (the neurotransmitter quantum), to compare the amplitudes of individual quantal responses between wild-type and *rab3A* mutant synapses (Fig. 1a). Both the amplitude of mEPSCs and their basal spontaneous rate are unchanged at the *rab3A*-mutant synapses (Fig. 1b).

With rapid repeated use, the average quantity of neurotransmitter released by a synapse declines, a decline that probably represents the depletion of fusion-competent vesicles in the 'readily releasable pool' at the presynaptic terminal⁵. This depletion is faster in *rab3A*-mutant neurons, indicating some alteration in the efficiency of synaptic vesicle trafficking⁶. To determine whether the depletion is faster because the readily releasable pool is smaller than usual, we compared the pool size at synapses formed between cultured hippocampal neurons prepared from wild-type or *rab3A*-mutant mice.

The size of the readily releasable pool was estimated by applying hypertonic solution which induces vesicular release through a Ca^{2+} -independent mechanism and sharply increases the rate of occurrence of mEPSCs⁷. When a synapse is placed in a hyperosmotic environment (solution made hyperosmolar with 0.5 M sucrose), quanta are at first released rapidly and then, as the readily releasable pool is depleted, at progressively slower rates (Fig. 2a). The total number of quanta released per synapse during this burst of exocytosis defines the readily releasable pool, whereas the steady-state exocytic rate corresponds to the balance of release and recruitment of new vesicles to the readily releasable pool. The pool size of readily releasable vesicles, determined by integrating the release transient (Fig. 1 legend), is not appreciably altered in *rab3A*-mutant cells. The result from a synaptotagmin I mutant is included for comparison (Fig. 2c). In contrast to the reduction in evoked quantal release caused by the lack of fast Ca^{2+} -dependent transmitter release⁸, Ca^{2+} -independent release induced by hypertonic solution is intact in synaptotagmin I mutant cells (releasable pool size is 25–36 vesicles), confirming the specific role for functional synaptotagmin I in Ca^{2+} -dependent fusion processes.

Figure 1 Spontaneous transmitter release is normal in the absence of Rab3A. Cumulative probability distributions of mean mEPSC amplitude (**a**) and frequency (**b**) from wild-type (straight line) and *rab3A* mutant (dotted line) neurons. Spontaneous mEPSCs were collected from hippocampal neurons grown in culture. The overall mean mEPSC amplitudes were 23 ± 3 pA ($\pm$ s.e.m.; $n = 14$) in wild-type cells, and 25 ± 2 pA ($\pm$ s.e.m.; $n = 14$) in mutant cells. The overall mean

Although the size of the release-ready vesicle pool in Rab3A-deficient mice is normal, a change in the kinetics of vesicle recruitment—such as vesicle docking and priming—could reflect slower refilling of the pool. We next examined such a possibility by estimating the refilling rate of the readily releasable pool in *rab3A*-mutant cultures with double-pulse application of hypertonic solution. The peak response to the second of a pair of hypertonic solution pulses applied at short interpulse intervals recovers with a time constant of ~ 7 s (ref. 7). This measure corresponds to the rate of replenishing releasable vesicles to a functionally defined pool that is shared by both nerve-impulse-evoked and hypertonic-solution-induced fusion processes⁹. As shown in Fig. 2d, the rate of recovery between successive applications of hypertonic solution is comparable for wild-type and *rab3A*-mutant neurons; *rab3A*-knockout mice are therefore not deficient in ensuring the continual supply of synaptic vesicles for fusion. Synaptotagmin I-mutant neurons also show an unaltered refilling rate of the readily releasable pool.

We have shown so far that the size and the refilling rate of the readily releasable vesicle pool are normal in *rab3A*-mutant mice, so

mEPSC frequencies were 0.62 ± 0.18 per min per synapse ($\pm$ s.e.m.; $n = 10$ neurons) in wild-type and 0.88 ± 0.20 per min per synapse ($\pm$ s.e.m.; $n = 20$ neurons) in mutant cells. The distributions between the wild type and Rab3A mutants of both mEPSC amplitude (**a**) and frequency (**b**) are not significantly different, as determined by Kolmogorov-Smirnov test ($P > 0.2$).

Figure 2 The size and refilling rate of the readily releasable pool are unchanged in *rab3A*-mutant synapses **a–c**, *rab3A*-mutant cells display a normal sized pool of release-ready vesicles. Average quantal release rate per synapse is shown as a function of time before and during local application of hypertonic solution (solid bar; external solution to which 0.5 M sucrose was added) for wild-type (**a**; $n = 6$), *rab3A*-mutant (**b**; $n = 5$), and synaptotagmin I (*sytl*)-mutant (**c**; $n = 3$) cells. Experiments were done in cultured hippocampal neurons. Bath solution contained 3 mM Ca^{2+} , 2 mM Mg^{2+} and 0.5 μM tetrodotoxin (TTX). Error bars represent s.e.m. There is no appreciable difference in the rate profile of quantal release induced by hypertonic solution among the three groups. The estimated releasable pool size was 26–35 vesicles in wild type, 28–31 vesicles in *rab3A* mutant, and 25–36 vesicles in synaptotagmin I mutant cells (see Methods). **d**, Recruitment of vesicles to the release-ready pool is not defective in *rab3A* mutant cells. Pairwise application of hypertonic solution was used to compare the recovery rates of the releasable pool of vesicles in cultured hippocampal neurons. Peak responses to the second hypertonic solution applied at various intervals are shown as a percentage of the peak response to first application. Points represented by wild-type cells (diamonds; $n = 11$), *rab3A*-mutant cells (circles; $n = 9$), and synaptotagmin-I-mutant cells (triangles; $n = 7$) have been fitted to an exponential recovery curve with a time constant of 6.9 s (solid line). Error bars indicate s.e.m. Wild-type, *rab3A*-mutant and synaptotagmin-I-mutant cultures show similar recovery rates of the releasable pool of vesicles.

the steps leading to the final Ca^{2+} -triggered exocytosis must be unaffected by the absence of *rab3A*. Moreover, the quantal properties are also unaffected by the mutation. The faster depletion with repeated stimuli in *rab3A* mutants⁶ could then be due to an increased efficiency of transmitter release⁵. We therefore tested whether synaptic vesicle fusion is affected in the mutant mice by comparing the quantity of transmitter released per synapse in wild-type and mutant neurons.

We monitored synaptic transmission from a known number of synapses in wild-type or *rab3A*-mutant hippocampal cultures to determine the average amount of transmitter release per synapse for every stimulus (Fig. 3a). A typical wild-type synapse releases, on average, about 0.5 quanta per stimulus under our recording conditions. In contrast, *rab3A*-mutant cells show a twofold increase in peak quantal release (one quantum per synapse per stimulation). Lack of Rab3A thus leads to an increase in the average number of vesicle fusions per synapse for every stimulus. Figure 3a also shows the average peak quantal release for synaptotagmin I mutant neurons for comparison. The greatly reduced efficiency of transmitter release in synaptotagmin I mutant cells, caused by disruption of the mechanisms supporting fast Ca^{2+} -dependent synaptic transmission, is reflected in the diminished peak quantal release per synapse relative to wild-type synapses⁸.

Although the average number of quanta released in response to nerve stimulation is increased, the size and refilling rate of the readily releasable pool, as well as the basal quantal release rate, remain normal in *rab3A*-mutant synapses. Contrary to the presumed role for Rab proteins in vesicle docking, Rab3A protein function therefore involves some step whose modification becomes apparent only at the time of evoked neurotransmitter release.

To determine whether Rab3A alters release properties in a time window immediately after stimulation, we tested the effect of Rab3A on a form of short-term plasticity that results from directly modulating Ca^{2+} -dependent exocytosis. Paired-pulse facilitation is an enhancement of the response to the second of a pair of stimuli given at short interpulse intervals (Fig. 3b, circles). This facilitation reflects an increase in release in response to the second stimulus caused by the previous exposure of the presynaptic terminal to a build-up of Ca^{2+} ions during the first pulse¹⁰. Facilitation is most prominent at shorter intervals, when the effects of Ca^{2+} accumula-

tion from the first stimulation have the strongest influence on transmitter release caused by the second pulse¹¹.

Facilitation at *rab3A*-mutant synapses is much larger than at wild-type synapses at short interpulse intervals (10–20 ms; Fig. 3b, triangles). The *rab3A*-mutant phenotype is most markedly expressed within 20 ms, starting with the onset of release to the first pulse, and is evident as increased release during this period. We have already shown that *rab3A*-mutant synapses release, on average, more quanta for every stimulus (Fig. 3a). The fact that *rab3A*-mutant synapses facilitate even more than expected when probed with a second closely spaced stimulus indicates that the mutant phenotype is enhanced by some aspect of the first release; otherwise, if mutant synapses simply release more quanta for every stimulus, then the ratio of the second response to the first response (that is, the paired-pulse facilitation ratio) should have remained unchanged.

We define transmitter-release probability, P , as the probability (reliability) that one or more quanta are released from a synapse in response to nerve stimulation. An increased quantal release in *rab3A*-mutant synapses could then reflect either an increase in P of releasing one or more quanta, and/or an increase in the number of quanta released when a release does occur (note that P remains unchanged in the latter case). We therefore compared the kinetics of inhibition of synaptic responses by MK-801 for wild-type and *rab3A*-mutant slices in two ways. First, we measured the probability of exocytosis for a given synapse population. Second, we repeated the experiment under conditions in which we could detect the difference in the amount of transmitter released for a synapse population.

MK-801 is effectively an irreversible open-channel blocker of the NMDA (*N*-methyl-D-aspartate) subtype of glutamate receptors¹². A progressive blocking of NMDA-receptor-mediated synaptic currents by MK-801 is used to estimate the transmitter-release probability P . For example, a higher value of P causes more synaptic vesicle fusions so that, on average, more transmitter molecules are released for each trial; under such conditions more synaptic NMDA receptors are opened for every stimulus, leading to a faster rate of inhibition of NMDA-receptor-mediated synaptic currents by MK-801 (refs 13, 14). Changes in the MK-801 blocking rate are evident with several-fold changes in release probability¹⁵. Consequently, if

Figure 3 Mutation in *rab3A* involves alterations closely related to the final step of exocytosis. **a**, Peak quantal release is selectively enhanced in *rab3A*-mutant cultures. Average peak quantal release per synapse was determined by monitoring synaptic transmission at 0.1 Hz from a restricted number of synapses through local microperfusion⁸. Culture preparation was used for this experiment. Peak quantal release (peak current per quantal size) was 0.48 ± 0.09 for wild-type cell pairs ($n = 14$), 1.12 ± 0.24 for *rab3A*-mutant neuron pairs ($n = 14$), and 0.086 ± 0.016 for synaptotagmin-I mutant cells pairs ($n = 7$)⁸. Whereas the lack of functional Ca^{2+} sensor causes a severe reduction in peak quantal release, the absence of Rab3A protein increases the amount of transmitter released per stimulus. Error bars indicate s.e.m. **b**, Paired-pulse facilitation is enhanced in *rab3A* mutants. EPSCs to pairs of stimuli were monitored in wild-type (circles: $n = 15$ –22 slices from 3–6 mice) and *rab3A*-mutant (triangles: $n = 16$ –26 slices from 4–7 mice) hippocampal slices in extracellular recording solution that contained 2.5 mM Ca^{2+} and 1.3 mM Mg^{2+} . The facilitation ratio is the ratio of the second response to the first response, and is shown as a function of interpulse interval. Error bars give s.e.m. Paired-pulse response is greater for *rab3A* mutant neurons at all interpulse intervals tested, and is seen consistently under all conditions that alter initial release probability—that is, in four different extracellular Ca^{2+} and Mg^{2+} concentrations (5, 2.5, 1.25 mM Ca^{2+} at 1.3 mM Mg^{2+} , and 1 mM Ca^{2+} /2.4 mM Mg^{2+} ; data not shown). A previous study of *rab3A*-knockout mice included an analysis of paired-pulse facilitation; however, paired-pulse responses were examined for longer interpulse intervals only (≥ 50 ms; Fig. 2b, ref. 6). We focused our analysis on shorter interpulse intervals, primarily between 6 and 20 ms, when the effect of *rab3A* mutation on paired-pulse facilitation is most prominent.

the twofold increase in peak release in *rab3A* mutants (Fig. 3a) is due to a twofold increase in P , then we should detect a faster MK-801 blocking rate in the mutants under normal recording conditions. However, the rates of MK-801 blocking are the same in wild-type and *rab3A*-mutant slices (Fig. 4a). The increase in quantal release in the *rab3A* mutants is therefore not caused simply by a general increase in P across all synapses.

As peak quantal release increases but MK-801 blocking rate is unaffected, transmitter release in the mutants might be raised non-uniformly across synapses. Specifically, increased vesicle fusion must occur at synapses that already release at least one quantum (that is, those synapses associated with a higher value of P) because fusion of a single quantum under ordinary conditions is believed to saturate postsynaptic NMDA receptors^{16,17}. The sensitivity to MK-801 would therefore be unaffected only when additional quanta are released at synapses—that is, if *rab3A* mutation causes an increase in the number of quanta released per synapse instead of a higher number of synapses that release a quantum. To increase the detection limit of postsynaptic NMDA receptors to released transmitters, we did the MK-801 experiments in the presence of a high-affinity competitive antagonist of the NMDA receptor, D-2-amino-5-phosphonovalerate (AP5)¹⁸. Addition of 5 μ M AP5 reduces the effective agonist concentration and thus decreases the number of open NMDA receptors available for MK-801 binding. Consequently, the MK-801 blocking rate in the presence of AP5 is greatly slowed in wild-type slices (circles in Fig. 4a and b, circles). Under these conditions, the increased release of glutamate should reduce the potency of the competitive antagonist, leading to more open NMDA receptors and resulting in faster MK-801 blocking. In the presence of AP5, we find that the MK-801 block is indeed faster in *rab3A*-mutant slices than in wild-type slices (Fig. 4b), consistent with increased quantal release in *rab3A*-mutant synapses (Fig. 3a). Moreover, the fact that MK-801 blocking is faster in *rab3A* mutants only in the presence of AP5 is in accord with the saturation of postsynaptic NMDA receptors under the normal conditions used for monitoring transmitter release. The different MK-801 blocking rates in the absence (unaltered) and presence (faster) of AP5 in *rab3A*-knockout mice relative to wild-type mice, are evidence for an increased number of quantal releases per synapse and not a general increase in the number of synapses that release a quantum.

We have shown that the average quantal release per synapse is increased in the absence of Rab3A. Rab3A, therefore, is normally involved in regulating the number of quanta released at hippocampal synapses. A similar role for Rab3A in regulating fusion in chromaffin cells has been proposed^{19,20}. We have also determined when Rab3A functions during the different stages of synaptic vesicle exocytosis. Contrary to the popular view that Rab proteins are involved in vesicle docking, the effect of the *rab3A* mutation is specific to the time of nerve-impulse-evoked vesicle fusion; processes leading to Ca^{2+} -triggered exocytosis remained unchanged. The specificity of Rab3A function for nerve-evoked release is supported by the finding that spontaneous release is normal and also by a previous report that Rab3A dissociates from synaptic vesicles upon exocytosis only if release is triggered by Ca^{2+} influx²¹. Although Rab3A functions in regulating vesicle fusion at the time of the Ca^{2+} signal, Rab3A and/or its possible effectors may become incorporated or act on the fusion complex at an earlier stage, before the influx of Ca^{2+} ions.

How is quantal release increased in the absence of Rab3A? We believe that this increase is not caused by a general increase in release probability, but by more vesicle fusions when a release does occur because (1) occlusion or attenuation of paired-pulse facilitation, which is expected of processes that rely on increasing P (refs 22–24), was not observed in the mutants; rather, at all interpulse intervals tested, *rab3A*-mutant slices showed greater facilitation than wild-type slices (Fig. 3b), compatible with an unaltered value of P in the absence of Rab3A; (2) the fact that a difference in MK-801 blocking rates between wild-type and mutant slices was revealed only in the presence of a competitive antagonist of NMDA receptors indicates that quantal release is increased without an overall increase in P in the mutants. In addition, the MK-801 results argue for the saturation of NMDA receptors during synaptic transmission under normal conditions. Altogether, unaltered P at *rab3A* mutant synapses suggests that Rab3A regulates the efficiency of the fusion process after activation of the Ca^{2+} -sensitive step that governs the release probability.

We propose a mechanism by which Rab3A might regulate vesicle fusion. Transmitter release at hippocampal synapses is believed to be limited to a single quantum, even under conditions of maximal release probability^{25–28}. We suggest that Rab3A plays a role in the

Figure 4 In the absence of Rab3A, the probability of transmitter release is not altered but the amount of transmitter released is increased. **a**, NMDA-receptor component of whole-cell synaptic currents was monitored in the presence of 5 μ M CNQX and 40 μ M MK-801 from wild-type (circles; $n = 6$ slices from two mice) and *rab3A*-mutant (triangles; $n = 7$ slices from two mice) hippocampal slices. The charge carried by the NMDA receptor currents was normalized to the intercept value obtained from linear regression of the first 11 responses, and is plotted as a function of stimulus number; synaptic responses were monitored every 10 s (ref. 15). Extracellular recording solution contained 2.5 mM Ca^{2+} and 1.3 mM Mg^{2+} . Error bars are s.e.m. The progressive blocking rate of NMDA-receptor-mediated synaptic currents by MK-801 is indistinguishable in wild-type and *rab3A* mutant cells, indicating that release probability is unaltered in the mutant animals. **b**, Comparison of the MK-801 blocking rate in the presence of 5 μ M APV reveals a faster rate in the mutant cells (triangles; $n = 10$ slices from five mice) relative to wild-type neurons (circles; $n = 10$ slices from three mice), consistent with increased quantal release at individual synapses in the absence of Rab3A.

mechanism that ordinarily limits release to a single quantum: suppose that such regulation works by raising the energy barrier for neighbouring vesicle fusion as a result of rapid mechanical deformation of the active zone, caused by insertion of additional membrane by the fused vesicle²⁹. Rab3A or its effectors could secure such rapid deformation of the active zone by facilitating the complete and efficient fusion of the synaptic vesicle membrane through destabilizing the metastable intermediate fusion complex. A role for temporal regulation of the stability of the docking and/or fusion complex has also been proposed for the endosome-specific Rab family member Rab5 (ref. 30). Then, in the absence of Rab3A, the precise steps are slowed that lead to complete fusion of the synaptic vesicle upon binding Ca^{2+} , retarding the warping of the active zone sufficiently to prevent the energy barrier for ensuing neighbouring vesicle fusion from increasing fast enough, allowing multiple releases to occur. Our model accounts for the fact that release probability is independent of Rab3A function. □

Methods

Culture experiments. Low-density microisland cultures of hippocampal pyramidal neurons were prepared from E15–E17 wild-type, *rab3A*-mutant and synaptotagmin I mutant mouse embryos as described⁸; five to seven embryos from the same mother were used for every wild-type and *rab3A* mutant culture preparation. Independent cultures of the same genotype yielded reproducible results, and each set of experiments was performed on separate cultures. Some of the experiments were also done on cultures prepared from rat (Fig. 2), and were included in the wild-type data as there were no differences in synaptic properties of the cultures of the two rodent species. Cells were used 8–13 days after plating; coverslips were changed after each recording so that the number of cells/cell pairs given in the figure legends represents the number of coverslips used. extracellular recording solution contained (in mM): NaCl 137, KCl 5, CaCl_2 10, MgCl_2 0.5, D-glucose 10, HEPES–NaOH (pH 7.3) 5, and 100 μM picrotoxin was also added; concentrations of MgCl_2 and CaCl_2 were varied when indicated. The internal recording solution was as before⁸. Recordings were made with axopatch 200 (Axon Instruments). Signals were filtered at 2 kHz, digitized at 5 kHz and analysed with programs written in AXOBASIC, VisualBASIC and C.

Hypertonic solution was applied locally as described⁷, and the quantal release rate was normalized for the number of synapses. The upper limit of the releasable pool size was estimated by the total number of quanta released per synapse during the first 4 s of hypertonic solution application; the lower limit was defined by subtracting the steady-state pool size for the 4-s period. The releasable pool size for wild-type cells determined here was slightly higher than a previous estimate⁷: the discrepancy is probably due to underestimation of the number of synapses in the perfused area, because, depending on the intensity threshold of the fluorescence staining of the synapses (determined visually after immunofluorescence labelling against synapsin I; ref. 8) used for defining synapses, the number of synapses can readily vary by at least 2-fold (C.E.S. and J. F. Wesseling, unpublished observations). The wild-type and mutant culture experiments were nevertheless done under identical conditions; we observed no difference in the pool size among the three groups.

Slice experiments. Acute transverse hippocampal slices were prepared and whole-cell recordings were made as described^{26–28}. Concentrations of CaCl_2 and MgCl_2 were varied as indicated for each experiment. 1 mM QX314 (Sigma) was included in the recording pipette to suppress spike generation in the post-synaptic cell. MK-801 and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) were from Research Biochemicals International.

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Correspondence should be addressed to Y.G. (e-mail: ygoda@jeeves.ucsd.edu).

C-terminal binding domain of Rho GDP-dissociation inhibitor directs N-terminal inhibitory peptide to GTPases

Yuying Q. Gosser*, Tyzoon K. Nomanbhoy†, Behzad Aghazadeh*‡, Danny Manor†, Carolyn Combs†, Richard A. Cerione† & Michael K. Rosen*

* Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021, USA

† Departments of Biochemistry, Molecular and Cell Biology, and Department of Pharmacology, Cornell University, Ithaca, New York 14853, USA

‡ Graduate Program in Physiology and Biophysics, Cornell University Graduate School of Medical Sciences, New York, New York 10021, USA

The Rho GDP-dissociation inhibitors (GDIs) negatively regulate Rho-family GTPases^{1,2}. The inhibitory activity of GDI derives both from an ability to bind the carboxy-terminal isoprene of Rho family members and extract them from membranes^{3,4}, and from inhibition of GTPase cycling between the GTP- and GDP-bound states^{4,5}. Here we demonstrate that these binding and inhibitory functions of rhoGDI can be attributed to two structurally