Shared Cortical Anatomy for Motor Awareness and Motor Control

A. Berti, *et al.*

Science **309**, 488 (2005);
DOI: 10.1126/science.1110625

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

Shared Cortical Anatomy for Motor Awareness and Motor Control

A. Berti,[1]* G. Bottini,[2] M. Gandola,[2] L. Pia,[1] N. Smania,[3] A. Stracciari,[4] I. Castiglioni,[5] G. Vallar,[6] E. Paulesu[6]

In everyday life, the successful monitoring of behavior requires continuous updating of the effectiveness of motor acts; one crucial step is becoming aware of the movements one is performing. We studied the anatomical distribution of lesions in right-brain–damaged hemiplegic patients, who obstinately denied their motor impairment, claiming that they could move their paralyzed limbs. Denial was associated with lesions in areas related to the programming of motor acts, particularly Brodmann's premotor areas 6 and 44, motor area 4, and the somatosensory cortex. This association suggests that monitoring systems may be implemented within the same cortical network that is responsible for the primary function that has to be monitored.

Although much of the functioning of the body's motor systems occurs without awareness, humans are aware that they are moving (or not moving) different parts of their body, even when performing automatic movements. They can also predict and be aware of the consequences of an intended motor act, with reference to their goals (*1*). There are, however, pathological states in which movement awareness is dramatically impaired (*2*, *3*). One instance of this phenomenon can be found in right-brain–damaged patients, affected by left-sided hemiplegia, who may deny their deficit and claim that their paralyzed limbs can still move. This disturbance has been termed anosognosia (*4*) or denial of motor deficit, and it has often been considered part of a multifaceted disorder of spatial representation and awareness, called unilateral neglect (*5*). However, because neglect and anosognosia may occur independently (*6*–*8*), their cognitive and possibly neuropathological bases might differ (*9*).

We compared the distributions of brain lesions in patients showing left spatial neglect, left hemiplegia, and anosognosia for motor deficit, with those of patients showing neglect, left hemiplegia, but not anosognosia. Any significant difference in brain damage between these groups of patients should correspond to the damage specific to anosognosia.

Thirty patients with a complete left motor deficit (hemiplegia) after unilateral right-

Table 1. Demographic and clinical data of the right-brain–damaged patients with and without anosognosia for hemiplegia. The ranges are reported in parentheses. The asterisk indicates the number of patients in whom tactile sensation was tested, with somatosensory deficit. n.t., not tested; A, anosognosia; N, neglect; + indicates present; – indicates absent.

Patient group	No.	Age	Education (years)	Somatosensory deficit of the contralesional side*	Line cancellation Albert test, mean no. of items crossed		Letter cancellation Diller test, percentage of items crossed		Bell test, mean no. of items crossed	
					Left	Right	Left	Right	Left	Right
A+N+	17	75.2 (36–89)	6.5 (0–13)	13/15 (86.7%)	4/18	12/18	0	14.8	0.6/17	3.2/17
A–N+	12	75.9 (62–85)	5.7 (4–13)	6/11 (54.5%)	8/18	15/18	8.6	65	1.8/17	7.7/17
A+N– (Patient RMA)	1	56	5	No somatosensory deficit	18/18	18/18	n.t.	n.t.	17/17	15/17

[1]Psychology Department and Center for Cognitive Science, University of Turin, Via Po 14, 10123 Turin, Italy. [2]Psychology Department, University of Pavia, Piazza Botta 6, 27100 Pavia, Italy. [3]Rehabilitation Unit, G.B. Rossi University Hospital, P. le L. A. Scuro, 37134 Verona, Italy. [4]Neurology Unit, S. Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy. [5]Bioimaging and Molecular Physiology Institute, Consiglio Nazionale delle Ricerche, Via Fratelli Cervi 93, 20090 Segrate, Milan, Italy. [6]Department of Psychology, Building U6, University of Milano-Bicocca, Piazza dell'Ateneo Nuovo 1, 20126 Milan, Italy.

*To whom correspondence should be addressed. E-mail: berti@psych.unito.it

Table 2. Brain lesion analysis. Results of anatomical statistical comparisons of patients with hemiplegia and anosognosia and patients with hemiplegia without anosognosia are shown. Stereotactic coordinates are distances, in millimeters, of the center of mass of a significant lesion, from the anterior commissure (*10*). The table also reports the results for the supramarginal gyrus [Broadmann's Area (BA) 40], the angular gyrus (BA 39), and the superior temporal gyrus (BA 22/42) associated with spatial neglect (*11*, *24*, *25*). These areas were not significantly more affected in either group of patients. DLPC, dorsolateral prefrontal cortex; dPMc, dorsal premotor cortex; IFGop, opercular inferior frontal gyrus; PostCG, post-central gyrus; PreCG, precentral gyrus; SMG, supramarginal gyrus; AG, angular gyrus; STG, superior temporal gyrus. n.a., not available, which indicated that it was technically not reliable to isolate the number of voxels damaged in the insula. ns, not significant.

Brain Region (Brodmann's area)	Patients with anosognosia	Patients without anosognosia	Overall regional chi-square *P* value	Mann-Whitney *P* value based on lesion voxel count	Stereotactic coordinates of voxels of maximal significance for each Brodmann's area			Voxel-wise chi-square *P* value
					X	*Y*	*Z*	
dPMc (6)	16/17	5/12	0.007	0.004	33	–2	43	0.025
IFGop (44)	15/17	4/12	0.007	0.0025	44	5	29	0.05
PostCG (3, 1, 2)	15/17	6/12	0.06	0.03	67	–18	37	0.01
PreCG (4)	14/17	4/12	0.021	0.012	43	–6	33	0.01
DLPC (46)	11/17	1/12	0.008	0.003	32	21	36	0.05
Insula	11/17	2/12	0.03	n.a.	49	4	11	0.05
SMG (40)	12/17	7/12	ns: 0.8	ns: 0.17	-	-	-	-
STG (22)	11/17	4/12	ns: 0.2	ns: 0.17	-	-	-	-
STG (42)	10/17	4/12	ns: 0.3	ns: 0.25	-	-	-	-
AG (39)	9/17	6/12	ns: >0.9	ns: 0.70	-	-	-	-
Deep centrum semiovale	0/17	7/12	0.001	n.a.	18	–20	21	0.05

sided brain lesions were investigated. Patients were grouped according to the presence or absence of anosognosia for left hemiplegia and left unilateral spatial neglect. Seventeen patients had anosognosia and neglect, and 12 had neglect without anosognosia (Table 1) (*10*). The superimposed lesion plots of the 17 hemiplegic patients with anosognosia (A+) and with neglect (N+) (Fig. 1A) were compared with the 12 hemiplegic patients without anosognosia (A–) and with neglect (N+) (Fig. 1B). Table 2 shows the areas that were most frequently involved in the anosognosic group and the statistical comparison between the two groups. The anatomical chi-square distribution of the comparison of A+N+ versus A–N+ patients is shown in Fig. 2. In anosognosic patients, the maximum overlap of brain lesion was centered on the dorsal premotor cortex (Brodmann's area 6; damaged in 94% of A+N+ patients), followed by area 44 and the somatosensory area (88% of the patients), and by the primary motor cortex (82% of the patients). Other neighboring structures that were differentially involved were area 46 and the insula. In both groups of patients, the inferior parietal lobule, which is traditionally associated with spatial neglect (*11*), was frequently involved. The reversed chi-square comparison of the distribution of the brain lesion in the A–N+ groups compared with the A+N+ group (Fig. 2 and Table 2) showed one single area of difference: the white matter, where axons of the corticospinal tract are located in the depth of the centrum semiovale. This suggest that A–N+ patients tend to have more subcortical lesions than do A+N+ patients, and less or no involvement of cortical areas, confirming that spared awareness of the motor deficit in patients A–N+ arises from the sparing of the premotor cerebral cortex, which is contrarily affected in A+N+ patients.

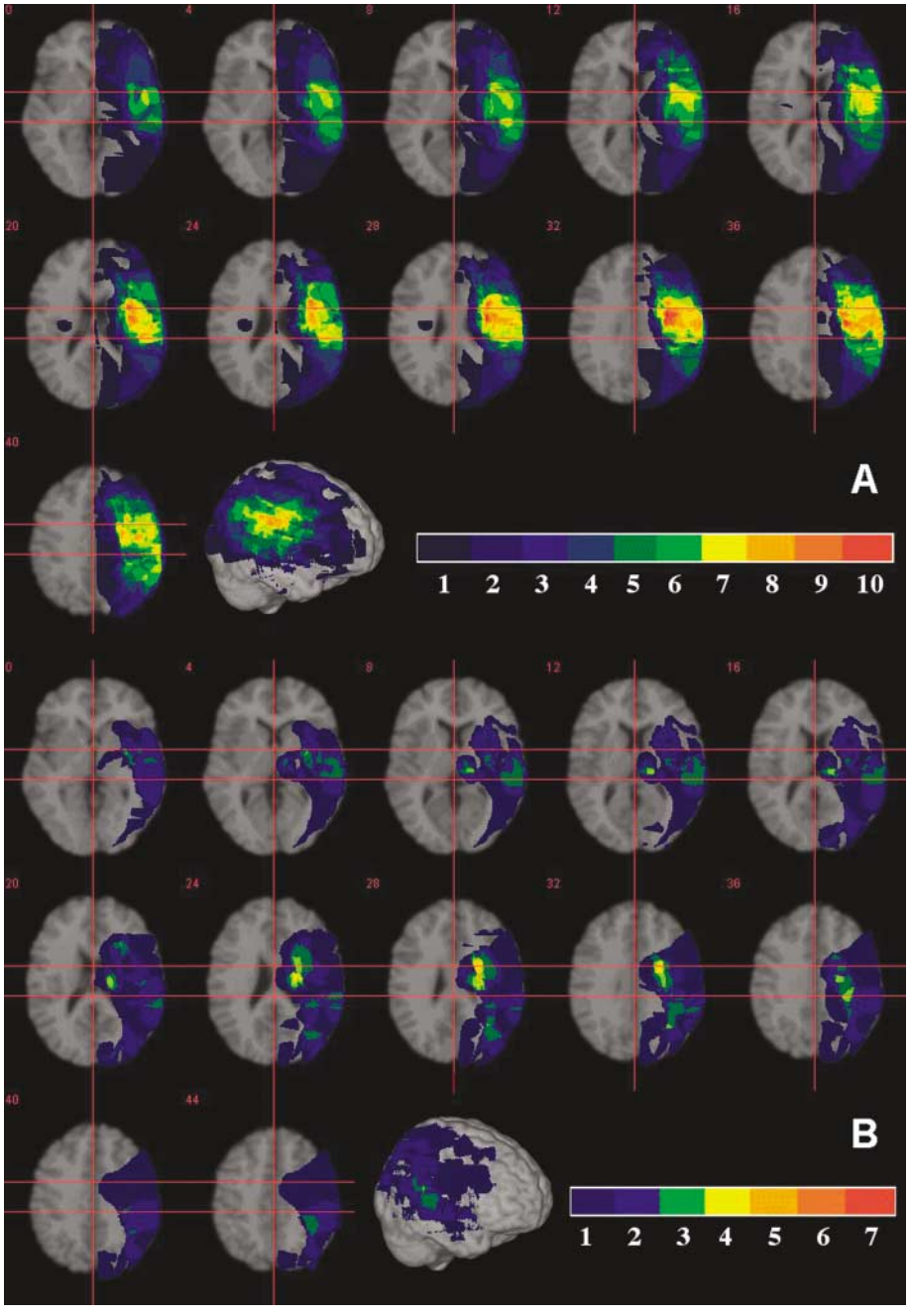

Fig. 1. (**A**) Regional lesion distribution in patients with hemiplegia, spatial neglect, and anosognosia. The regional frequency of brain lesions in each area of the right hemisphere is expressed according to the color scale (for example, areas in red show that a lesion is present in 10 patients). (**B**) Regional lesion distribution in patients with hemiplegia, spatial neglect, and no anosognosia. Each individual lesion has been superimposed onto a standard brain conforming to stereotactic space.

From the above statistical comparisons, having controlled for the factor "spatial neglect," we predicted that patients with pure anosognosia (i.e., patients showing the denial symptom without neglect) should present with a brain damage that largely overlaps with the areas that distinguish the A+N+ group from the A–N+ patients. We found such a patient (patient RMA in Table 1) who had a pure form of anosognosia without spatial neglect. Figure 3 shows the overlap between his brain damage and the areas that were significantly more affected in the A+N+ group, as compared with the A–N+ group. The predicted overlap is evident in areas 6, 4, 44, and 3 and in the insula.

These findings permit us to tease apart the neural correlates of denial of motor deficit from the parietotemporal network commonly associated with spatial neglect; anosognosia for hemiplegia is best explained by the involvement of motor and premotor areas (particularly area 6) and also, although less frequently, of prefrontal areas, such as area 46, and the insula. Observations in patient RMA, who has a pure form of anosognosia, also indicate that the frontal lesions did not affect prefrontal areas but mainly involved the frontal agranular cortex, including area 4, the dorsolateral portion of area 6, and area 44. These latter areas are fundamental components of circuits related to the programming of motor acts, both in humans and in monkeys (*12*, *13*). Premotor areas, and also the primary motor cortex, activate not only during motor preparation but also during motor imagery (*1*, *14*), and a large body of psychological and neuroimaging experiments have been interpreted as favoring a functional equivalence between action generation, action simulation, action verbalization, and perception of action (*15*). Moreover, even the interpretation of others' actions is related to the activity of neurons located in the premotor cortex (area 6) (*16*, *17*). Our data expand this

knowledge by providing evidence of an involvement of the premotor frontal regions in the conscious monitoring of motor acts. These findings are directly relevant for models of motor control and, more generally, for accounts of consciousness. Indeed, the involvement of premotor areas in self-monitoring of action implies that, at least for motor functions, monitoring is neither the prerogative of some kind of central executive system, hierarchically superimposed on sensory-motor and cognitive functions (*18*, *19*), nor a function that is physiologically and anatomically separated from the primary process that has to be monitored. Instead, the anatomical correlates of anosognosia show that monitoring can be implemented in the same neural network responsible for the process that has to be controlled. This is in keeping with other findings in the domain of altered self-monitoring (*20*).

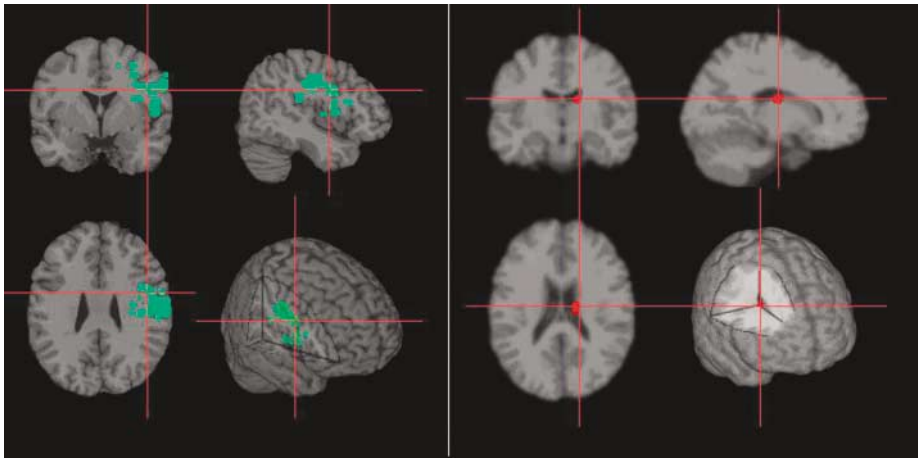

Fig. 2. Anatomical comparison between ansognosic and nonansognosic patients. On the left, brain areas more frequently damaged in patients with neglect, left hemiplegia, and anosognosia are shown as identified by a pixelwise chi-square analysis. On the right, brain areas more frequently damaged in patients with neglect, left hemiplegia, and without anosognosia are shown. Methods, stereotactic coordinates, and levels of significance are described in Table 2 (*10*).

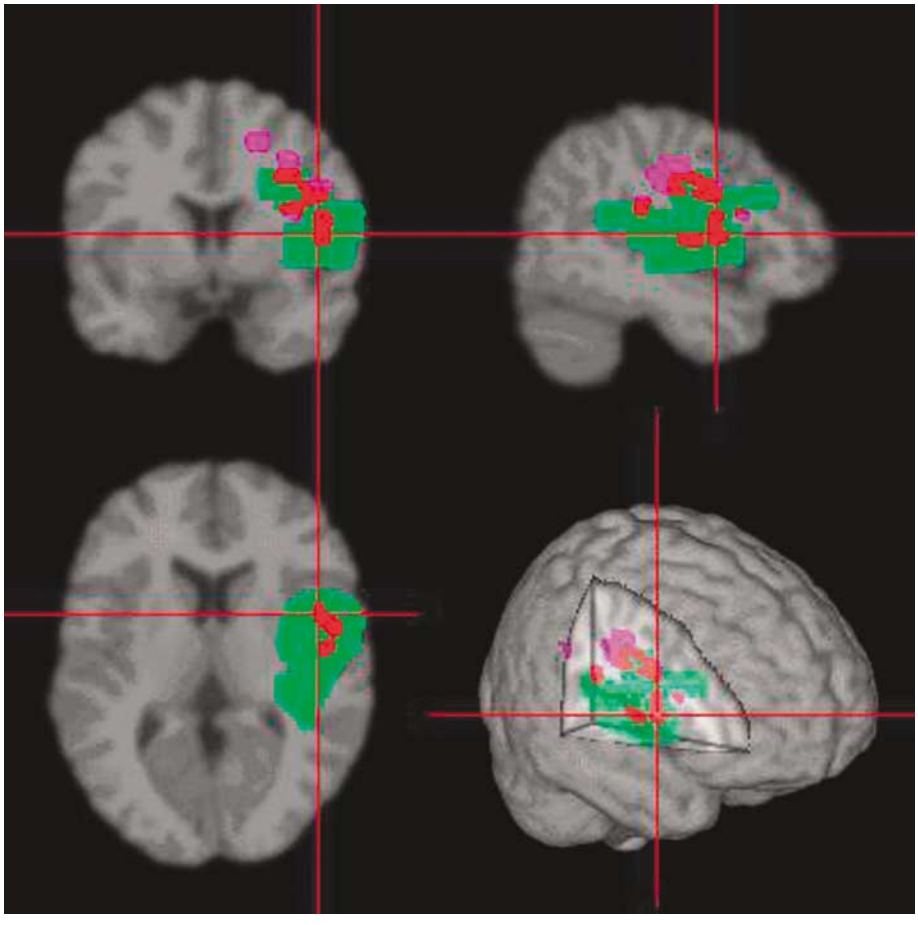

Fig. 3. Brain lesion in patient RMA, who has pure anosognosia for hemiplegia without spatial neglect. Areas in red are in common with the brain areas identified by the pixelwise chi-square comparison of patients with anosognosia, as opposed to those without anosognosia. Areas in green are brain areas damaged in patient RMA that are not identified by the chi-square analysis. Areas in purple are those identified by the chi-square analysis and not damaged in patient RMA.

Recently, it has been proposed that the denial behavior might be due to the fact that patients may experience the movement they intended to perform, but are not able to distinguish between a purely simulated (imaged) action and the actual status of the motor system (*3*, *21*). Our data show that some parts of the motor system can be spared in anosognosic patients. We may speculate that, although the damage to premotor areas impairs the motor-monitoring process, it is still possible, because of some spared premotor activity, to generate a distorted representation of the intended motor act, which is responsible for the false belief of being able to move. Also in this view, the experience of intention to move does not depend on the functioning of a single cortical region, but instead arises from a dynamic interaction between different premotor areas.

Finally, because movements occur in egocentric space, the close association often observed between left-sided anosognosia with left-sided neglect may reflect the damage to common components of a frontoparietal network, specifically related to spatiomotor integration. The lesion to a single component of this network gives rise to selective and spatially constrained disorders of awareness (*22*, *23*).

References and Notes

1. M. Jeannerod, *Behav. Brain Sci.* **17**, 187 (1994).
2. E. Bisiach, G. Geminiani, in *Awareness of Deficit After Brain Injury*, G. P. Prigatano, D. L. Schacter, Eds. (Oxford Univ. Press, New York, 1991), pp. 17–39.
3. C. D. Frith, S. J. Blakemore, D. M. Wolpert, *Philos. Trans. R. Soc. London Ser. B* **355**, 1771 (2000).
4. J. Babinski, *Rev. Neurol.* **27**, 845 (1914).
5. E. Bisiach, in *Handbook of Clinical and Experimental Neuropsychology*, G. Denes, L. Pizzamiglio, Eds. (Psychology Press, Hove, UK, 1999), pp. 479–493.
6. A. Berti, E. Ladavas, M. Della Corte, *J. Int. Neuropsychol. Soc.* **2**, 426 (1996).
7. E. Bisiach, G. Vallar, D. Perani, C. Papagno, A. Berti, *Neuropsychologia* **24**, 471 (1986).
8. V. Dauriac-Le Masson *et al.*, *Rev. Neurol.* **158**, 427 (2002).
9. M. Small, S. Ellis, *Eur. Neurol.* **36**, 353 (1996).
10. Materials and methods are available as supporting material on *Science* Online.
11. G. Vallar, D. Perani, *Neuropsychologia* **24**, 609 (1986).
12. G. Rizzolatti, G. Luppino, M. Matelli, *Electroencephalogr. Clin. Neurophysiol.* **106**, 283 (1998).
13. J. Tanji, *Curr. Opin. Neurobiol.* **6**, 782 (1996).
14. M. Roth *et al.*, *Neuroreport* **7**, 1280 (1996).
15. J. Grezes, J. Decety, *Hum. Brain Mapp.* **12**, 1 (2001).
16. P. L. Jackson, J. Decety, *Curr. Opin. Neurobiol.* **14**, 259 (2004).

17. G. Rizzolatti, L. Craighero, *Annu. Rev. Neurosci.* **27**, 169 (2004).
18. E. Bisiach, A. Berti, in *The Cognitive Neurosciences*, M. S. Gazzaniga, Ed. (MIT Press, Cambridge, MA, 1995), pp. 1131–1340.
19. G. Bottini *et al.*, *Nature* **376**, 778 (1995).
20. P. K. McGuire *et al.*, *Br. J. Psychiatry* **169**, 148 (1996).
21. A. Berti, E. Làdavas, A. Stracciari, C. Giannarelli, A. Ossola, *Cognit. Neuropsych.* **3**, 21 (1998).
22. A. Berti, G. Rizzolatti, *J. Cogn. Neurosci.* **4**, 345 (1992).
23. L. Pia, M. Neppi-Modona, R. Ricci, A. Berti, *Cortex* **40**, 367 (2004).
24. H. O. Karnath, S. Ferber, M. Himmelbach, *Nature* **411**, 950 (2001).
25. D. J. Mort *et al.*, *Brain* **126**, 1986 (2003).
26. This work was supported by Ministero dell'Istruzione, dell'Università e della Ricerca Prin grants to A.B., G.V., and E.P, and by a Bracco spa grant to G.B. and E.P.

Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5733/488/DC1
Materials and Methods
References

3 February 2005; accepted 17 May 2005
10.1126/science.1110625

Distinct Kinetic Changes in Neurotransmitter Release After SNARE Protein Cleavage

Takeshi Sakaba,* Alexander Stein, Reinhard Jahn, Erwin Neher*

Neurotransmitter release is triggered by calcium ions and depends critically on the correct function of three types of SNARE [soluble *N*-ethylmaleimide–sensitive factor attachment protein (SNAP) receptor] proteins. With use of the large calyx of Held presynaptic terminal from rats, we found that cleavage of different SNARE proteins by clostridial neurotoxins caused distinct kinetic changes in neurotransmitter release. When elevating calcium ion concentration directly at the presynaptic terminal with the use of caged calcium, cleavage of SNAP-25 by botulinum toxin A (BoNT/A) produced a strong reduction in the calcium sensitivity for release, whereas cleavage of syntaxin using BoNT/C1 and synaptobrevin using tetanus toxin (TeNT) produced an all-or-nothing block without changing the kinetics of remaining vesicles. When stimulating release by calcium influx through channels, a difference between BoNT/C1 and TeNT emerged, which suggests that cleavage of synaptobrevin modifies the coupling between channels and release-competent vesicles.

Clostridial neurotoxins, which cleave SNARE [soluble *N*-ethylmaleimide–sensitive factor (NSF) attachment protein (SNAP) receptor] proteins (*1*), block Ca^{2+}-dependent neurotransmitter release (*2*). Distinct kinetic differences in their action (*3*, *4*) indicate that it matters which of the SNAREs is cleaved and at what particular site (Fig. 1A). However, studies disagree in their mechanistic interpretations regarding toxin action (*3*–*9*). Conventional synapses allow only limited manipulations at the presynaptic terminal, rendering it difficult to discern which steps between Ca^{2+} entry and transmitter release are impaired by a given toxin. The calyx of Held, a large glutamatergic nerve terminal in the auditory pathway, can be voltage-clamped (*10*, *11*); the intracellular Ca^{2+} concentration ($[Ca^{2+}]$) can be manipulated by caged Ca^{2+} as well as by controlled Ca^{2+} influx (*12*, *13*); and recombinantly produced light chains of the toxins can be introduced directly into the terminal. This allows for testing toxin action acutely, applying stimuli of graded strength, and monitoring Ca^{2+} influx. By using these possibilities, we uncovered remarkable differences in the action of toxins.

Presynaptic terminals were stimulated by voltage-clamp depolarization, and two toxins cleaving either syntaxin (BoNT/C1) (Fig. 1B) or SNAP-25 (BoNT/A) (Fig. 1C) were infused by a patch pipette (*14*). In each case, a pulse protocol consisting of 10 action potential–like (AP-like) depolarizations followed by a 50-ms depolarization was repeatedly applied to the presynaptic terminal. The presynaptic Ca^{2+} current did not change appreciably during the 10-min recording period (89 ± 7% and 91 ± 5%) (top traces, Fig. 1, B and C). The excitatory postsynaptic current (EPSC), however, changed strongly in this time interval (middle traces).

The earliest records (blue) were taken at a time when toxin action was still modest. Similar to control, the EPSCs evoked by AP-like pulses displayed facilitation during the first two to three stimuli, followed by depression. Subsequent long-lasting depolarizations elicited large EPSCs, which were sufficient to release all remaining vesicles of the releasable pool (RP) (*15*, *16*). The pattern of change during toxin action was simplest for the action of BoNT/C1 (Fig. 1B). About 10 min after the start of toxin infusion, only small postsynaptic currents were observed during both the initial 10 AP-like pulses and the long-lasting depolarization. At an intermediate time (red trace, Fig. 1B), EPSCs were substantially reduced both for AP-like and long depolarizations. The sequence of facilitation and depression was preserved throughout the time course of the experiment (Fig. 1B right). The pattern of change was strikingly different for BoNT/A (Fig. 1C): At about 10 min of toxin action, the responses to the AP-like pulses were completely blocked, whereas cumulative release elicited by the long depolarization was still almost intact (88 ± 9.0%, $n = 5$). Furthermore, at an intermediate time (7 min) responses during the AP-like pulses facilitated more strongly (Fig. 1C right).

The gradual and uniform decrease observed under BoNT/C1 is compatible with an all-or-nothing block of release sites, whereas the distinct kinetic changes induced by BoNT/A call for other mechanisms of action. Further characterization of the mechanisms is difficult to achieve by voltage-clamp experiments alone, because elevation of intracellular $[Ca^{2+}]$ through Ca^{2+} channels is spatially not homogenous, and different vesicles may be exposed to different $[Ca^{2+}]$ signals (*17*, *18*). Ca^{2+} uncaging circumvents this problem by elevating $[Ca^{2+}]$ uniformly within the presynaptic terminal. We infused a mixture of the caged-Ca^{2+} compound DM-Nitrophen (Calbiochem, Bad Soden, Germany) and the Ca^{2+} indicator dye Fura 2FF (TEFLABS, Austin, TX) into the cell together with toxins and rapidly elevated $[Ca^{2+}]$ by an ultraviolet flash to around 10 μM (Fig. 2). This $[Ca^{2+}]$ is within the range postulated to occur during nerve-evoked action potentials (*12*, *13*). Comparing control (Fig. 2A) with BoNT/C1 (Fig. 2B), BoNT/A (Fig. 2C), and a third toxin, tetanus toxin (TeNT) (Fig. 2D), which cleaves synaptobrevin. The absolute magnitudes of the EPSCs were found to be smaller under the influence of toxins. In all cases, the flash was followed after 60 ms by a long-lasting depolarization. At 8 min of toxin infusion, the total number of vesicles released shortly after the flash was 3120 ± 348 vesicles under control conditions and 1347 ± 258 vesicles and 995 ± 161 vesicles under BoNT/C1 and TeNT, respectively. However, the vesicles that escaped toxin action were released with a time course similar to that of control for both BoNT/C1 and TeNT (*14*). Subsequent depolarization evoked little further release. In contrast, the step-like elevation of $[Ca^{2+}]$ to 10 μM elicited only a

Department of Neurobiology and Department of Membrane Biophysics, Max Planck Institute for Biophysical Chemistry, Göttingen 37077, Germany.

*To whom correspondence should be addressed. E-mail: eneher@gwdg.de (E.N.); tsakaba@gwdg.de (T.S.)