Dynamics of Depolarization and Hyperpolarization in the Frontal Cortex and Saccade Goal

Eyal Seidemann,* Amos Arieli, Amiram Grinvald, Hamutal Slovin

The frontal eye field and neighboring area 8Ar of the primate cortex are involved in programming and execution of saccades. Electrical microstimulation in these regions elicits short-latency contralateral saccades. To determine how spatiotemporal dynamics of microstimulation-evoked activity are converted into saccade plans, we used a combination of real-time optical imaging and microstimulation in behaving monkeys. Short stimulation trains evoked a rapid and widespread wave of depolarization followed by unexpected large and prolonged hyperpolarization. During this hyperpolarization saccades are almost exclusively ipsilateral, suggesting an important role for hyperpolarization in determining saccade goal.

Electrical stimulation has been used extensively in brain research since its introduction more than 100 years ago (*1*). Despite the popularity of this technique and its potential clinical applications (*2*, *3*), many questions remain regarding the spatial profile and the temporal dynamics of the neural response that is evoked by intracortical microstimulation (*4*, *5*). One of the first cortical regions that was systematically explored using microstimulation is the frontal eye field (FEF) (*6*–*8*), a cortical area situated in the anterior bank of the arcuate sulcus and known to be involved in planning and execution of saccadic eye movements (*9*–*11*). The FEF and neighboring area 8Ar (*12*) contain a representation of saccades to the contralateral hemifield. Microstimulation in these regions evokes short-latency conjugate saccades that depend on the stimulation site and are very similar to voluntary saccades (*6*–*8*).

We sought to measure and characterize directly the neural response that is elicited by microstimulation in the frontal cortex of the behaving monkey and to determine the relation of this response to the saccadic eye movements that are evoked by stimulation. We thus combined standard electrophysiological techniques in awake behaving monkeys with optical imaging using voltage-sensitive dyes (*13*). This combination provides a powerful paradigm for measuring the electrical activity of populations of neurons in the brain of behaving monkeys at excellent spatial and temporal resolutions (*14*). The dye signal measures the sum of the membrane-potential changes in all the neuronal elements in the imaged area, emphasizing subthreshold synaptic potentials in neuronal arborizations originating from neurons in all cortical layers whose dendrites reach the superficial cortical layers (*15*).

Combined microstimulation (*16*) and optical imaging were applied to the frontal cortex in four hemispheres of two monkeys at a total of 39 recording sites (*17*) while the monkey performed a simple fixation task (*18*). To image the FEF and 8Ar, we opened a cranial window over the arcuate sulcus, then removed the dura and replaced it with a transparent artificial dura (*19*). The imaged/stimulated area included primarily a narrow strip of cortex, lying 1 to 3 mm anterior to the arcuate sulcus, from which short-latency contralateral saccades could be evoked (*16*, *20*). Figure 1A shows the frontal cortex in the left hemisphere of one monkey 3 weeks after surgery; the arcuate sulcus (Ar) and the principal sulcus (Pr) can be seen through the transparent dura. We stained the cortex with blue voltage-sensitive dyes and imaged the resulting activation pattern at up to 400 frames per second (*13*).

The activation pattern evoked by microstimulation at one stimulation site is shown in Fig. 1B. A train of stimulation pulses (50 μA, 500 Hz) was applied for 24 ms at the site marked by X. Shortly after stimulation onset there was a large and widespread increase in fluorescence, which corresponds to an overall depolarization. This depolarization spread over a large cortical area in a rather uniform manner, consistent with the spread of activity measured in the visual cortex (*21*, *22*). Following this brief depolarization, the response dropped sharply, and most of the imaged area became dark. This large and rapid drop in the optical signal corresponds to an overall hyperpolarization of the average membrane potential of the neuronal population to a level well below baseline (*23*).

The mean time course of the optical signal for four different stimulation conditions was measured in a small area 0.4 mm by 0.4 mm near the site of the stimulating electrode (Fig. 2). A short stimulation train of 24 ms (green curve) elicited a rapid depolarization that was followed by a large and prolonged hyperpolarization. The hyperpolarization returned to baseline about 230 ms after stimulation onset and showed a small depolarization overshoot. When stimulation was applied for a longer period of 80 ms (dark blue curve), the response still peaked at about 25 ms and then dropped to a lower depolarization level despite the continuation of the stimulation. When the train of stimulation was divided into four short trains with short gaps in between (red and cyan curves), the response

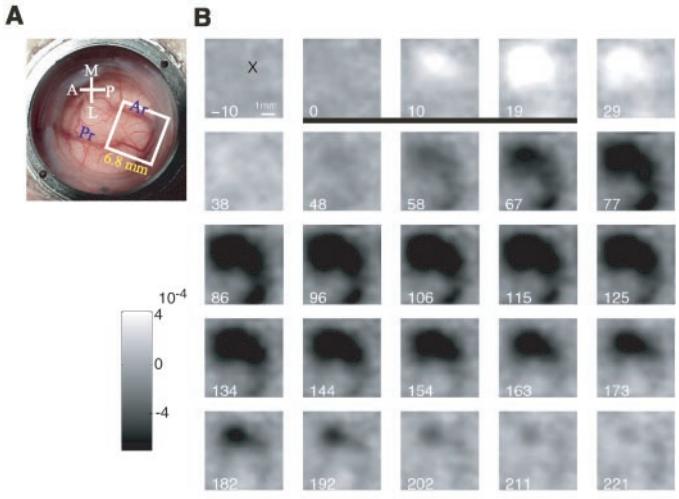

Fig. 1. Spatiotemporal dynamics of microstimulation-evoked activity. (**A**) The FEF in the left hemisphere as seen through the cranial window and the transparent artificial dura. The dorsomedial arm of the arcuate sulcus (Ar) can be seen, as well as the principal sulcus (Pr). The area imaged in the experiment shown in (B) is marked by a white rectangle. (**B**) Sequence of images of the cortex during microstimulation. The time interval between frames is 9.6 ms. The imaged area is 6.8 mm by 6.8 mm. Stimulation is applied through frames 2 to 4 and is indicated by the black horizontal line. The site of penetration of the stimulating electrode is marked by an X. Stimulation at this site evoked a 30° saccade to the right. The optical signals were low pass–filtered (σ = 250 μm) for display purposes only. The activation pattern is averaged across 30 repetitions.

Department of Neurobiology and the Grodetsky Center for Studies of Higher Brain Function, Weizmann Institute of Science, Rehovot 76100, Israel.

*To whom correspondence should be addressed. E-mail: eyal.seidemann@weizmann.ac.il

went through four cycles of depolarization and hyperpolarization. This large hyperpolarization was typical for most stimulation sites in the frontal cortex. In contrast, in the primary visual cortex of the awake monkey, the same stimulation parameters do not evoke similar hyperpolarization (*22*).

The time course of the optical signal shows that each depolarization response in the frontal cortex is followed by a similar, and sometimes even larger, hyperpolarization response. How does this interplay between depolarization and hyperpolarization affect the saccades that are elicited by microstimulation? The horizontal eye velocity traces from three trials in which eye movements were evoked by stimulation at a site in the right frontal cortex that represents a 20° saccade to the left are shown in Fig. 3A. At this site and with these stimulation parameters there was a large variability in saccade initiation time, and in these three trials saccades were initiated 50, 80, and 100 ms after stimulation onset. Although in all three trials saccades were evoked under identical stimulation conditions, these saccades differ markedly. The early contralateral saccade (red curve) displayed the maximal amplitude and peak velocity characteristic for this site and was followed 130 ms later by a large ipsilateral saccade. The saccade that started around stimulation offset (green curve) displayed a smaller amplitude and reversed to the ipsilateral direction at midflight. The saccade that started about 20 ms after stimulation offset (cyan curve) was short and ipsiversive. All of these eye movements were evoked by the stimulation; on randomly interleaved trials without stimulation, the monkeys made no saccades during this interval.

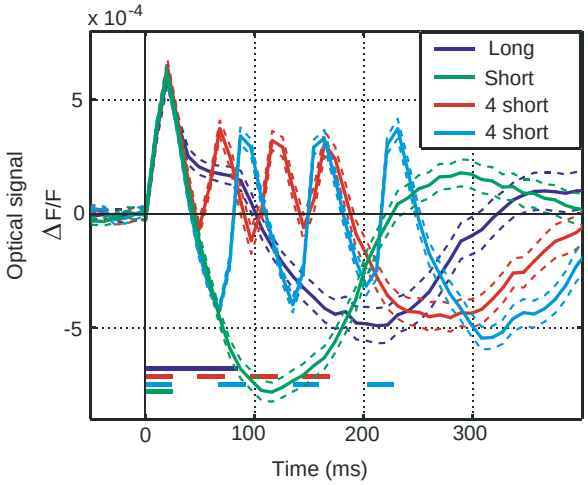

Fig. 2. Time course of the response to microstimulation. The curves show the mean ± SEM of the response to four stimulation conditions in a 0.4 mm by 0.4 mm area of cortex near the site of stimulation, measured from the same site as in Fig. 1. The number of repetitions in each condition is between 27 and 30. The horizontal lines at the bottom indicate the stimulation time for each stimulation condition.

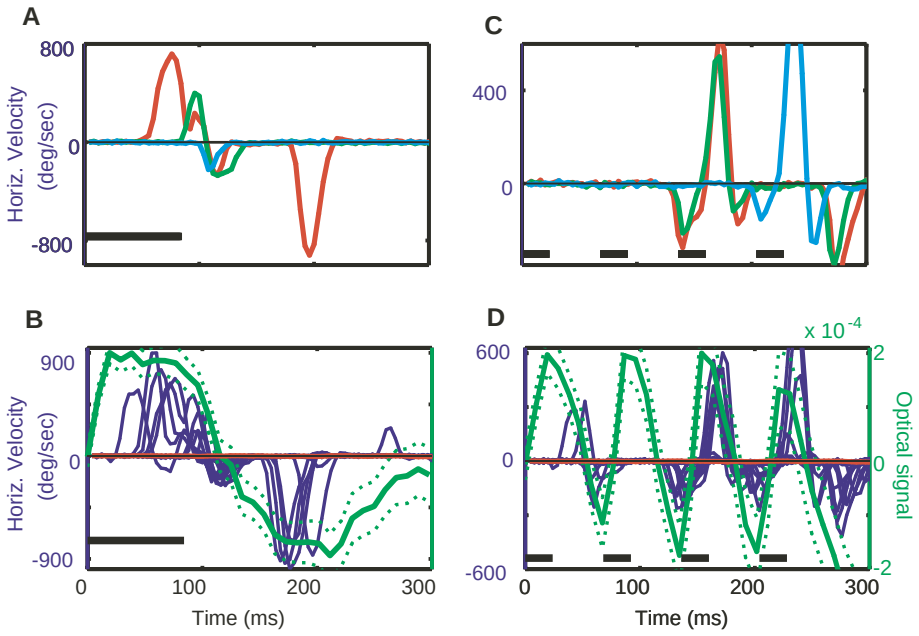

Fig. 3. Examples of the correlation between the properties of stimulation-evoked saccades and the response elicited by microstimulation. (**A** and **C**) Horizontal eye velocity traces for three trials with 80-ms stimulation (A) or four trains of 24-ms stimulation separated by an interval of 44 ms without stimulation (C). The horizontal lines at the bottom of each panel indicate stimulation times. (**B** and **D**) Comparison of the mean ± SEM of the time course of the optical signal (green curves) measured in a 0.4 mm by 0.4 mm area of cortex near the site of stimulation, and individual traces of horizontal eye velocity (blue curves) for long stimulation (B) or four short trains (D). Red curves indicate the mean ± SEM of horizontal eye velocity on nonstimulated trials (n = 52).

The changes in saccade properties over time closely parallel the time course of the optical signal measured near the stimulation site. The mean time course of the optical signal (green curve) and the horizontal eye velocity traces (blue curves) for all the trials at this site and at these stimulation parameters (n = 25 trials) are shown in Fig. 3B. Saccades that were initiated during strong depolarization were contralateral and displayed high peak velocity. Saccade peak velocity decreased during the drop in depolarization and then reversed to the ipsilateral direction. Saccades that were initiated during strong hyperpolarization were almost always large return saccades in the ipsilateral direction.

The correlation between the measured response and the properties of the evoked saccades holds also for other stimulation conditions. Figure 3C shows eye movements that were evoked during three trials of stimulation with four short trains at the same site as in Fig. 3, A and B. In these trials, the first short train failed to evoke a short-latency saccade. The first eye movement evoked in those trials was a short ipsiversive saccade rather than the large contraversive saccade typical for this site. In two trials (red and green curves), ipsiversive saccades were initiated about 35 ms after the offset of the second stimulation train, during the initial phase of hyperpolarization. During this ipsiversive saccade the third train was applied and evoked a large contraversive saccade. It was followed by another small ipsiversive saccade that coincided with another period of hyperpolarization. In the third trial (cyan curve), an ipsiversive saccade was initiated about 40 ms after the offset of the third train and showed the same sequence of ipsi-contra-ipsi movements.

Figure 3D compares the horizontal eye velocity traces (blue curves) and the mean optical response (green curve) for all trials with the same stimulation condition as in Fig. 3C (n = 26). The direction and peak horizontal eye velocities of the evoked saccades are highly correlated with the optical signal measured at the time of saccade initiation. The first two cycles of depolarization evoked almost no saccades. Saccades started preferentially during the hyperpolarization that followed the second or the third train and were almost always ipsiversive. During the periods

of depolarization following the third and fourth trains the eyes moved toward the contralateral direction; during the hyperpolarization intervals following the second, third, and fourth trains the eyes moved toward the ipsilateral direction.

Following microstimulation, the level of depolarization or hyperpolarization in FEF and 8Ar at the time of saccade initiation proves to be an excellent predictor of the direction, size, and peak velocity of the evoked saccades across our entire data set. The results obtained from 39 stimulation sites and 1044 stimulation-evoked saccades are summarized in Fig. 4 (*24*). Each panel shows a scatter plot of normalized saccade peak horizontal velocity against saccade initiation time. For this analysis we combined all saccades that occurred shortly after stimulation, irrespective of whether these were the first

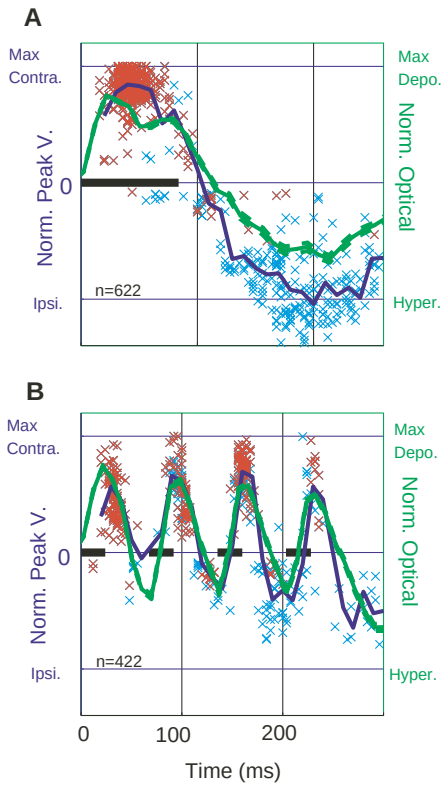

Fig. 4. Correlation between saccade peak velocity and the electrical response elicited by stimulation. (**A**) Long stimulation train. (**B**) Four short trains. The green curve shows the mean ± SEM of the normalized time course of the optical signal measured at all sites in which a reliable optical signal was obtained (*17*). The scatter plot of x's indicates the normalized peak horizontal eye velocity versus initiation time for all saccades obtained during this interval in all stimulation sites (*24*). Red x's indicate the first saccade evoked on a given trial, and cyan x's indicate later saccades. The blue line depicts the running average of the normalized peak horizontal eye velocities (in a bin of 10 ms). All of these saccades were taken from a period in which no saccades occurred during control trials without stimulation.

(red) or later saccades within a given trial (cyan). Under the two stimulation protocols there is a strong correlation between the measured optical signal and the properties of the evoked saccades. Saccades that were initiated during depolarization were almost exclusively contraversive. Saccades that were initiated during a period of hyperpolarization were almost exclusively ipsiversive. Saccades that were initiated when the absolute level of the response was high tended to have a high peak velocity and a large amplitude; saccades that started when the response was low tended to have a low peak velocity and a small amplitude (*25*). The correlation between the mean optical signal (green curve) and peak horizontal eye velocity of individual saccades (red and cyan x's) is highly significant for both stimulation conditions [$r = 0.93$ for continuous stimulation and $r = 0.82$ for four short trains; $P < 10^{-6}$ for both conditions (*26*)].

Our findings raise two questions regarding the hyperpolarization. First, what is the role of hyperpolarization? Hyperpolarization could mediate saccade target selection through competitive interactions between sites that represent alternative saccades (*27*). It could also serve as a reset mechanism necessary for normal saccade sequencing (*9*). Once a saccade of the desired amplitude and direction had been executed, activity that encodes this saccade should rapidly terminate so that another similar saccade would not be initiated erroneously. Indeed, many FEF neurons with presaccadic activity are actively suppressed following a saccade into their response field (*28*, *29*), suggesting that hyperpolarization may occur also after voluntary saccades. Hyperpolarization could also facilitate a rapid return saccade that would bring the eyes back to the previous eye position after desired and undesired saccades. Such rapid "glances" also occur during natural visual scanning behavior. A second question concerns the source of the hyperpolarization. In many experiments we found that hyperpolarization and depolarization started from different locations and displayed different spatial profiles (e.g., Fig. 1B). These results suggest that part of the hyperpolarization is caused by a stimulation-evoked recurrent inhibitory signal rather than by the well-known after-hyperpolarization. The finding that during hyperpolarization saccades are preferentially ipsilateral suggests that some of the delayed inhibitory signals could be mediated by "push-pull" inhibitory interactions between sites that encode opposite saccades in the two hemispheres (*30*). An exciting possibility, therefore, is that when the stimulation site is hyperpolarized, the site that encodes the opposite saccade in the other hemisphere is depolarized, contributing to the ipsilateral saccade.

Finally, our results demonstrate that neural activity with complex spatiotemporal dynamics can be elicited by microstimulation; these dynamics depend on the stimulated area and can have important behavioral correlates. These findings emphasize the importance of further characterization of microstimulation-evoked activity for the interpretation of the behavioral effects of microstimulation. Our finding that during hyperpolarization saccades are almost exclusively ipsilateral imposes new constraints on models of the neural mechanisms that compute saccade goal. Specifically, our results suggest that suppression of neural activity at a certain location in the saccade motor map in the frontal cortex is interpreted by downstream saccade circuitry as a signal in favor of an ipsilateral saccade.

References and Notes

1. D. Ferrier, *Philos. R. Soc. London* (*Biol.*) **165**, 433 (1875).
2. W. H. Dobelle, D. O. Quest, J. L. Antunes, T. S. Roberts, J. P. Girvin, *Neurosurgery* **5**, 521 (1979).
3. E. M. Schmidt *et al.*, *Brain* **119**, 507 (1996).
4. J. B. Ranck Jr., *Brain Res.* **98**, 417 (1975).
5. E. J. Tehovnik, *J. Neurosci. Methods* **65**, 1 (1996).
6. D. A. Robinson, A. F Fuchs *J. Neurophysiol.* **32**, 637 (1969).
7. P. H. Schiller, J. H. Sandell, *Exp. Brain. Res.* **49**, 381 (1983).
8. C. J. Bruce, M. E. Goldberg, M. C. Bushnell, G. B. Stanton, *J. Neurophysiol.* **54**, 714 (1985).
9. C. J. Bruce, in *Signal and Sense: Local and Global Order in Perceptual Maps*, G. M. Edelman, W. E. Gall, W. M. Cowan, Eds. (Wiley, New York, 1990), pp. 261–314.
10. J. D. Schall, in *Cerebral Cortex,* K. Rockland, A. Peters, J. Kaas, Eds. (Plenum, New York, 1997), vol. 12, pp. 527–638.
11. E. J. Tehovnik, M. A. Sommer, I. H. Chou, W. M. Slocum, P. H. Schiller, *Brain Res. Rev.* **32**, 413 (2000).
12. T. M. Preuss, P. S. Goldman-Rakic, *J. Comp. Neurol.* **310**, 475 (1991).
13. D. Shoham *et al*, *Neuron* **24**, 791 (1999).
14. Slovin *et al.* [H. Slovin, A. Arieli, R. Hildesheim, A. Grinvald, *Soc. Neurosci. Abstr.* **25**, 784 (1999); *Eur. J. Neurosc.* **12**, 126 (2000)] showed that it is possible to perform long-term voltage-sensitive dye imaging in behaving monkeys, repeatedly from the same cortical area, over a period of up to 1 year.
15. A. Grinvald *et al.*, in *Modern Techniques in Neuroscience Research*, U. Windhorst, H. Johansson, Eds. (Springer-Verlag, Berlin, Heidelberg, 1999), pp. 893–969.
16. Biphasic current pulses (500 Hz; 30 to 100 μA) were applied through standard tungsten microelectrodes. At each site we used the lowest current that yielded a reliable optical signal and evoked saccades (≤50 μA in most sites). These currents were around behavioral threshold for evoking saccades when the monkey was actively fixating, but clearly above threshold when the monkey was freely viewing, consistent with previous findings (*31*). Only sites from which clear, short-latency contralateral saccades could be evoked during active fixation were included in our study.
17. Saccades were evoked in 39 sites tested in a total of 21 optical imaging sessions. Of these sites, four were excluded from analysis of saccadic eye movements due to poor fixation. In 22 of the 39 sites, a clear response to microstimulation could be measured optically. Failure to obtain a reliable optical signal in the presence of a clear behavioral effect was due to poor staining. Experiments with no reliable optical signal were excluded from the analysis of the average evoked response. All of the results described here remain the same in the subset of 18 sites for which

reliable measurements of both optical and behavioral signals were obtained simultaneously (*26*).
18. The monkey was required to maintain fixation within 2° of a spot of light (0.1° by 0.1°) for 4 to 5 s to receive a reward (a drop of juice). Stimulation was applied in 66 to 80% of the trials. Only on stimulation trials could the monkey break fixation before the fixation spot disappeared (after stimulation onset) and still receive the reward. The fixation point disappeared immediately after the monkey broke fixation. In 77% of the stimulation sites the fixation point was positioned at the center of the screen; in the remaining sites it was positioned between 5° and 13° from the center of the screen toward the ipsilateral direction. Eye position was monitored by an infrared eye tracker (provided by Dr. Bouis Devices, Karlsruhe, Germany) and sampled at 250 Hz.
19. E. Shtoyerman, A. Arieli, H. Slovin, I. Vanzetta, A. Grinvald, *J. Neurosci.* **20**, 8111 (2000).
20. Most stimulation sites were located in the superficial cortical layers (first 700 μm). The spatiotemporal dynamics in response to microstimulation in the deeper layers was similar to that seen in response to stimulation in the superficial layers, consistent with previous in vitro imaging studies (*32*, *33*).
21. E. Seidemann, D. E. Glaser, A. Arieli, A. Grinvald, *Soc. Neurosci. Abstr*. **25**, 784 (1999).
22. E. Seidemann, H. Slovin, A. Arieli, A. Grinvald, *Soc. Neurosci. Abstr*. **26,** 1075 (2000).
23. The fast signals that we measure (both depolarization and hyperpolarization) are present only in the wavelength of fluorescence emission. Thus, these signals are not likely to be contaminated by mechanical artifacts or fast intrinsic signals. In addition, it has been shown recently, using intracellular recording in vivo, that the dye signal measures membrane-potential changes precisely [see figure 23 in (*15*); similar results were obtained in a recent in vitro study using the same dye (*34*)]. Furthermore, an in vitro study had previously demonstrated that microstimulation-evoked activity measured using real-time optical imaging is highly correlated with neural activity measured intracellularly (*32*).
24. Eye movements that had the typical bell-shaped velocity profile and peak velocity above 100°/s were considered saccades. In most stimulation sites the major component of the evoked saccade was horizontal; we therefore focused our quantitative analysis on the horizontal component of the evoked saccades (but see supplementary figures). To combine saccade peak velocity measurements across experiments (Fig. 4), we first normalized the peak velocity of each saccade relative to the maximal peak velocity obtained under all stimulation conditions at that site. The peak velocity of saccades in the contralateral direction was defined as positive, and in the ipsilateral direction as negative. Similarly, the average optical signal for each stimulation site was first normalized relative to the maximal depolarization observed at this site across all stimulation conditions before being combined to yield the grand mean shown in Fig. 4.
25. The properties of stimulation-evoked saccades observed here are consistent with those observed in previous studies (*6–8*, *31*, *35*). In particular, Stanford *et al*. (*35*) found that, following a short stimulation train in the superior colliculus, saccade amplitude becomes shorter with increasing saccade latency. No other study, to our knowledge, reported the existence of short ipsilateral saccades that occur during early hyperpolarization. This may not be surprising given that these saccades are comparatively rare (5% of the first saccades in Fig. 4) and, therefore, could easily have been overlooked by researchers who did not have access to the time course of depolarization and hyperpolarization. FEF stimulation can evoke ipsilateral saccades in the "colliding saccade" paradigm (*36*), when microstimulation is applied during an ongoing natural saccade. It remains to be determined whether the observed hyperpolarization plays a role also in the "colliding saccade" effect.
26. A correlation was computed between the normalized peak horizontal velocity for individual saccades (red and cyan x's in Fig. 4) and the mean normalized optical response at the time of saccade initiation (green curve in Fig. 4). To compute the correlation coefficient (Pearson's *r*), we paired each saccade peak velocity with the level of the average optical signal at the time of saccade initiation. The statistical significance of each correlation coefficient was assessed using an *F* test with 1 and $(n - 2)$ degrees of freedom, where *n* is the number of evoked saccades. The high correlation observed in Fig. 4 remains unaffected if we (i) select only the stimulation sites for which both behavioral and optical measurements were obtained simultaneously (*17*); (ii) select only stimulation sites in which stimulation current was ≤50 μA; (iii) select only stimulation sites that were likely to fall within the FEF; (iv) compute the correlation between the optical signals and the normalized saccade amplitude (see supplementary figures); or (v) compute the correlation between the optical signals and the normalized peak vertical eye velocity for saccades with a large vertical component (*37*).
27. J. D. Schall, *Rev. Neurosci.* **6**, 63 (1995).
28. C. J. Bruce, M. E. Goldberg, *J. Neurophysiol*. **53**, 603 (1985).
29. M. E. Goldberg, C. J. Bruce, *J. Neurophysiol*. **64**, 489 (1990).
30. J. Schlag, P. Dassonville, M. Schlag-Rey, *J. Neurophysiol*. **79**, 64 (1998).
31. M. E. Goldberg, M. C. Bushnell, C. J. Bruce, *Exp. Brain Res.* **61**, 579 (1986).
32. B. Albowitz, U. Kuhnt, *Eur. J. Neurosci.* **5**, 1349 (1993).
33. M. Tanifuji, T. Sugiyama, K. Murase, *Science* **266**, 1057 (1994).
34. C. C. H. Petersen, B. Sakmann, A. Grinvald, *Soc. Neurosci. Abstr*. **27** (2001).
35. T. R. Stanford, E. G. Freedman, D. L. Sparks, *J. Neurophysiol*. **76**, 3360 (1996).
36. Reviewed in J. Schlag, M. Schlag-Rey, *Trends. Neurosci.* **13**, 410 (1990).
37. Supplementary figures are available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5556/862/DC1.
38. All experimental procedures were in accordance with institutional and NIH regulations. We thank E. Ahisar, S. Barash, R. Born, C. J. Bruce, U. Zohary, M. E. Goldberg, and D. L. Sparks for critical comments on earlier versions of this manuscript and E. Tsabary, T. Eliahu, and D. Ettner for technical assistance. E.S. was supported by a Koshland Scholar award. This work was supported by grants from Abisch-Frankel (E.S. and A.G.) and the Gorodetsky Center and by the Koerber, Margaret Enoch, and Goldsmith foundations.

27 September 2001; accepted 13 December 2001

Chaperone Suppression of α-Synuclein Toxicity in a *Drosophila* Model for Parkinson's Disease

Pavan K. Auluck,[1] H. Y. Edwin Chan,[2]* John Q. Trojanowski,[3] Virginia M.-Y. Lee,[3] Nancy M. Bonini[1,2,4]†

Parkinson's disease is a movement disorder characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta. Dopaminergic neuronal loss also occurs in *Drosophila melanogaster* upon directed expression of α-synuclein, a protein implicated in the pathogenesis of Parkinson's disease and a major component of proteinaceous Lewy bodies. We report that directed expression of the molecular chaperone Hsp70 prevented dopaminergic neuronal loss associated with α-synuclein in *Drosophila* and that interference with endogenous chaperone activity accelerated α-synuclein toxicity. Furthermore, Lewy bodies in human postmortem tissue immunostained for molecular chaperones, also suggesting that chaperones may play a role in Parkinson's disease progression.

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is associated with resting tremor, postural rigidity, and progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Characteristic pathological features of PD include Lewy bodies (LBs), which are juxtanuclear ubiquitinated proteinaceous inclusions in neuronal perikarya, and Lewy neurites (LNs), which are similar protein aggregates found in neuronal processes (*1*). LBs and LNs are also characteristic of other neurodegenerative diseases, including the LB variant of Alzheimer's disease (LBVAD) and dementia with LBs (DLB). α-synuclein is a major constituent of inclusions found in these disorders, known as synucleinopathies (*2–4*). Moreover, two missense mutations in the gene encoding α-synuclein are linked to dominantly inherited PD, thereby directly implicating α-synuclein in disease pathogenesis (*5, 6*).

In *Drosophila*, directed expression of α-synuclein induces selective and progressive loss of dopaminergic neurons, as well as formation of α-synuclein–positive perinuclear and neuritic filamentous inclusions similar to LBs and LNs (*7*). Inclusion

[1]Department of Neuroscience, [2]Department of Biology, [3]Center for Neurodegenerative Disease Research and Department of Pathology and Laboratory Medicine, and [4]Howard Hughes Medical Institute, University of Pennsylvania and University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

*Present address: Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong. †To whom correspondence should be addressed. E-mail: nbonini@sas.upenn.edu