relation between p320 and mammalian high molecular weight MAPs is suggested by the observation that antibody against hog brain MAP 2 reacts weakly with trypanosomal p320, and vice versa (*20*). The only MAP whose complete sequence has been published so far is the tau protein of mouse brain (*29*). No similarity between p320 and tau primary sequence is observed. However, the tau sequence also contains three repetitive stretches of 18 amino acids. This, together with proteolysis experiments with mammalian MAP 3 (*30*) and direct protein sequencing of a 180-kD MAP from the spindle apparatus (*31*) suggest that a repetitive motive may be a common feature in MAP architecture.

REFERENCES AND NOTES

1. J. B. Olmsted, *Annu. Rev. Cell Biol.* **2**, 421 (1986).
2. L. Pillus and F. Solomon, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2468 (1986).
3. L. S. Goldstein, R. A. Laymon, R. J. McIntosh, *J. Cell Biol.* **102**, 2076 (1986).
4. G. Wiche, *Trends Biochem. Sci.* **10**, 67 (1985).
5. R. B. Vallee, *Cell Muscle Motility* **5**, 289 (1984).
6. A. Schneider *et al.*, *Cell Biol.* **104**, 431 (1987).
7. E. Angelopoulos, *J. Protozool.* **17**, 39 (1970).
8. J. Wu and L. R. Yarbrough, *Gene* **61**, 51 (1987).
9. M. A. Imboden, P. Laird, M. Affolter, T. Seebeck, *Nucleic Acids Res.* **15**, 7357 (1987).
10. S. Sather and N. Agabian, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5695 (1985).
11. M. A. Imboden, B. Blum, T. De Lange, R. Braun, T. Seebeck, *J. Mol. Biol.* **188**, 393 (1986).
12. B. E. Kimmel *et al.*, *Gene* **35**, 237 (1985).
13. L. S. Thomashow *et al.*, *Cell* **32**, 35 (1983).
14. T. Seebeck *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4634 (1983).
15. T. Sherwin *et al.*, *J. Cell Biol.* **104**, 439 (1987).
16. M. T. Dolan, C. G. Reid, H. P. Voorheis, *J. Cell Sci.* **80**, 123 (1986).
17. D. G. Russell, D. Miller, K. Gull, *Mol. Cell. Biol.* **4**, 779 (1984).
18. J. Stieger *et al.*, *J. Biol. Chem.* **259**, 4596 (1984).
19. T. Seebeck, V. Küng, T. Wyler, M. Müller, *Pro. Natl. Acad. Sci. U.S.A.* **85**, 1101 (1988); G. T. Bramblett, S. Chang, M. Flavin, *ibid.* **84**, 3259 (1987); C. G. Jensen and B. W. Smaill, *Ann. N.Y. Acad. Sci.* **466**, 417 (1986).
20. A. Hemphill *et al.*, in preparation.
21. A. Schneider, unpublished data.
22. J. B. Olmsted, *J. Biol. Chem.* **256**, 11955 (1981).

22a. V. Kueng, A. Schlaeppi, T. Seebeck, in preparation.

23. M. R. Mowatt and C. E. Clayton, *Mol. Cell. Biol.* 7, 2828 (1987).
24. I. Roditi *et al.*, *Nature* **325**, 272 (1987).
25. D. S. Peterson, R. A. Wrightsman, J. E. Manning, *ibid.* **322**, 566 (1986).
26. A. F. Cowman *et al.*, *Cell* **40**, 775 (1985).
27. M. A. T. Muskawitch and D. S. Nogness, *ibid.* **29**, 1041 (1982).
28. D. W. Speicher and V. Marchesi, *Nature* **311**, 177 (1984).
29. G. Lee, N. Cowan, M. Kirschner, *Science* **239**, 285 (1988).
30. G. Huber, D. Alaimo-Beuret, A. Matus, *J. Cell Biol.* **100**, 496 (1985).
31. G. Maier *et al.*, *Biol. Chem. Hoppe-Seyler* **368**, 440 (1987).
32. W. Herzog and K. Weber, *Eur. J. Biochem.* **92**, 1 (1987).
33. A. Fellous, J. Frances, A. M. Lemmon, J. Nunez, *Eur. J. Biochem.* **78**, 167 (1977).

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Nonoxidative Glucose Consumption During Focal Physiologic Neural Activity

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Brain glucose uptake, oxygen metabolism, and blood flow in humans were measured with positron emission tomography, and a resting-state molar ratio of oxygen to glucose consumption of 4.1:1 was obtained. Physiological neural activity, however, increased glucose uptake and blood flow much more (51 and 50 percent, respectively) than oxygen consumption (5 percent) and produced a molar ratio for the increases of 0.4:1. Transient increases in neural activity cause a tissue uptake of glucose in excess of that consumed by oxidative metabolism, acutely consume much less energy than previously believed, and regulate local blood flow for purposes other than oxidative metabolism.

UNDER NORMAL CONDITIONS, THE brain's energy demands [adenosine triphosphate (ATP) production] are thought to be provided almost exclusively by glucose oxidation. For the resting state, this idea is well established (*1*). More than 90% of all resting-state glucose consumption is oxidative, with 5% (or less) being metabolized to lactate. Because of the efficiency of oxidation (at least 15 times more ATP yield than glycolysis), more than 99% of the ATP production in resting tissue is by glucose oxidation. The phasic energy demands accompanying physiological increases in neural activity, however, are less well studied. Local cerebral glucose metabolic rate (CMRglu) and cerebral blood flow (CBF) are greatly increased by focal increases in neural activity. The increase in CMRglu has been thought to indicate a local increase in glucose oxidation, supporting large energy expenditures required to maintain membrane ionic gradients. The increase in local CBF has been considered a response to substrate (O_2) depletion and metabolite (CO_2) excess.

Challenging the conventional formulation, we reported that a focal, physiological increase in neural activity induced by peripheral tactile stimulation increased cerebral metabolic rate for O_2 ($CMRO_2$) minimally (5%), despite a large increase (29%) in local CBF (*2*). To confirm and extend these observations we measured the CMRglu, $CMRO_2$, and CBF of human visual cortex at rest and during visual stimulation.

All measurements were doubly paired; each volunteer (*3*) had blood flow and metabolic rate measured in both the stimulated and the unstimulated state during a single scanning session. The CBF and $CMRO_2$ were measured in one five-subject group (*4*); CBF and CMRglu were measured in a second five-subject group (*5*). Control-state measurements were made with the subject's eyes closed. Stimulated-state measurements were made as the subject viewed an annular reversing checkerboard (*6*). Cortical responses in primary visual cortex were identified with images of regional change (Figs. 1 and 2) created by superposition and subtraction (stimulus minus control) of intrasubject pairs of images for each subject and each variable.

In the resting state, the mean whole-brain (*7*) $CMRO_2$ and CMRglu were 1.50 ± 0.071 (SD) and 0.37 ± 0.053 μmol min^{-1} 100 g^{-1}, respectively. This is a 4.1:1 molar ratio, in good agreement with published values (*1, 8*). The same resting-state molar ratio (4.1:1) was present in primary visual cortex, where $CMRO_2$ and CMRglu were 1.71 ± 0.183 and 0.42 ± 0.033 μmol min^{-1} 100 g^{-1}, respectively. As expected, a strong resting-state regional correlation between CBF and metabolism ($CMRO_2$ and CMRglu) was evident from simple visual inspection of their regional distributions (Figs. 1 and 2). Moreover, multiregional (*7*) correlations of $CMRO_2$ and CBF ($n = 5$) and of CMRglu and CBF ($n = 5$) were significant ($P < 0.0001$) in all cases.

In every subject the CMRglu and CBF of visual cortex were markedly increased by visual stimulation (Table 1), rising a mean of 0.21 μmol min^{-1} 100 g^{-1} (51%) and 27 ml min^{-1} 100 g^{-1} (50%), respectively (Table 1 and Fig. 1). However, $CMRO_2$ increased only a mean of 0.08 μmol min^{-1} 100 g^{-1} (5%) (Table 1 and Fig. 2), in accord with our observations in somatosensory cortex (*2, 9*). The molar ratio for the increase in metabolic rate was only 0.4:1 (O_2:glucose). Thus, 91% of the activity-induced increase in glucose uptake was not oxidized.

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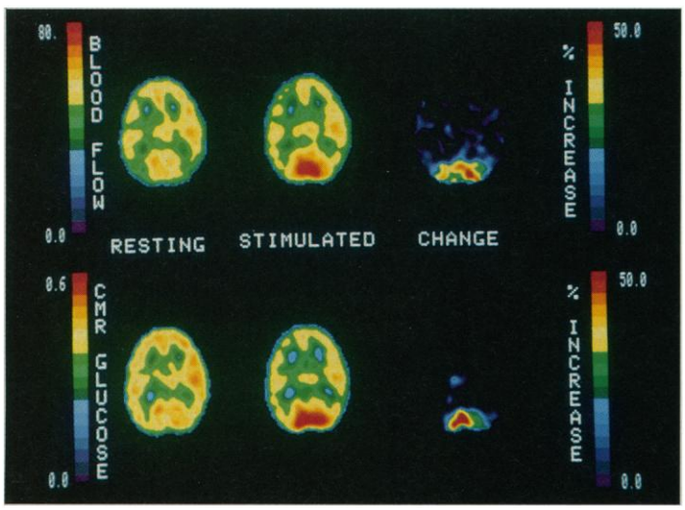
Observations have suggested that $CMRO_2$ is minimally increased by transient neural activation. In both this work and our study in somatosensory cortex (*2*), the increase in CBF so far exceeded the small increase in $CMRO_2$ that a highly significant decline in the extracted fraction of available O_2 (OEF) occurred (Table 1). That is, tissue Po_2 must have increased during phasic neural activity. Penfield noted that local tissue oxygenation (judged by the color of the venous effluent) increased, rather than decreased, during spontaneous focal seizures in human cerebral cortex (*10*). Direct measurement of venous O_2 tension during seizures in both animals and humans confirmed this impression (*11*). Cooper *et al.* found that a variety of simple motor and sensory tasks produced prompt, dramatic, and highly localized increases in cortical O_2 availability (*12*). Rapid eye-movement (REM) sleep also causes large increases in CBF (*13*) and CMRglu (*14*), but a decrease in OEF (*15*).

In contrast to the minimal increase in O_2 utilization, we found that glucose uptake rose dramatically during phasic neural activity, with a percentage change equivalent to the CBF change (Table 1 and Fig. 1). Similarly, physiological activation of sensorimotor cortex caused proportionate increases of CBF and CMRglu (*16*), but a disproportionately small increase in $CMRO_2$ (*2*). For glucose metabolism to rise in excess of O_2 consumption, lactate production (glycolysis) must increase. This increase has been directly corroborated by two independent methods. Using tissue fluoroscopy, Hossman and Linn (*17*) noted an increase in tissue lactate in rat somatosensory cortex during forepaw stimulation. Prichard *et al.* (*18*) used magnetic resonance spectroscopy to demonstrate an increase in tissue lactate of rabbit visual cortex during optic nerve stimulation.

Despite the large increase in glucose uptake observed here, the actual acute energy yield must be quite small. If one assumes that the entire increase in O_2 uptake was consumed by glucose oxidation at a 4.1:1 ratio and that all the remaining glucose was metabolized to lactate, the maximum possible increase in energy (ATP) production is only 8%. If a considerable fraction of the increase in glucose uptake is incorporated into glycogen (*19, 20*), the acute energy demands of neural activity must be even less than 8%. Creutzfeldt reached a similar conclusion by computing the energy demands of neural activity from heat production and estimating that "only about 0.3 to 3.0%, or even less, of the cortical energy consumption can be accounted for by spike activity of cortical nerve cells" (*21*, p. 25). A doubling of neural electrical activity, therefore, should increase $CMRO_2$ by 6% or less, in agreement with the small $CMRO_2$ changes that we have observed. Similarly, Van den Berg calculated by enzymatic capacity that glucose oxidation is at (or near) maximal capacity at rest and that "increases of glucose oxidation during stimulation, whether natural or experimental, cannot therefore take

Table 1. Stimulus-induced changes in human visual cortex. Values for CBF and $CMRO_2$ (*4*), OEF, and CMRglu (*5*) refer to absolute changes (stimulated state minus resting state) induced in visual cortex by a reversing checkerboard stimulus. Percentage changes were computed as stimulated state minus resting state divided by resting state times 100. CBF is expressed in milliliters per minute per 100 g of brain tissue. $CMRO_2$ and CMGglu are expressed in micromoles per minute per 100 g of brain tissue. OEF (O_2 extracted fraction) is the regional O_2 consumption (metabolism to H_2O) expressed as a fraction of the total O_2 delivered by the blood. *P* is the significance level by paired *t* test of the resting- versus stimulated-state value of each variable. The region of interest was 13.5 mm by 13.5 mm and centered over the zone of maximal CBF change (Figs. 1 and 2). *P* values were as follows: CBF, <0.000001; $CMRO_2$, = 0.4; OEF, <0.002; and CMRglu, <0.0003.

Scan	CBF	%	$CMRO_2$	%	OEF	%	CMRglu	Percent
1161	20.8	39					0.18	41
1178	16.3	30					0.18	42
1189	30.4	59					0.25	66
1191	31.0	47					0.25	56
1194	25.2	63					0.19	49
1216	40.7	76	0.29	16	−0.20	−33		
1228	28.3	49	0.09	5	−0.17	−30		
1232	29.0	46	−0.23	−12	−0.24	−40		
1238	20.5	34	0.11	8	−0.09	−20		
1241	28.8	53	0.14	8	−0.17	−33		
Mean	27.1	49.6	0.08	5.0	−0.17	−31.2	0.21	50.8
SD	6.85		0.19		0.06		0.037	



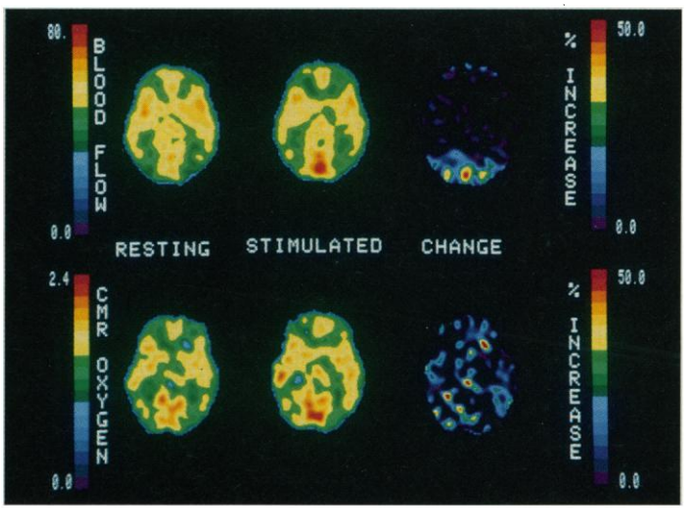


Fig. 1 (left). Glucose metabolic rate (**lower row**) (*5*) and blood flow (**upper row**) (*4*) were closely coupled throughout the brain both at rest and during visual stimulation. Phasic neural activation (visual stimulation) increased regional glucose uptake and blood flow by similar amounts (56 and 48%, respectively). These images are from a single scanning session (subject 1191 in Table 1) and pass through the same brain plane. This subject's regional changes were those closest to the group mean (Table 1) and were not the most dramatic responses. **Fig. 2 (right).** The metabolic rate of O_2 (**lower row**) (*4*) and blood flow (**upper row**) (*4*) were closely coupled throughout the brain at rest. Phasic neural activation (visual stimulation), however, increased regional O_2 consumption minimally (5%) while markedly increasing blood flow (49%). These images are from a single scanning session (subject 1228 in Table 1) and pass through the same brain plane. This subject's regional changes were those closest to the group mean (Table 1) and were not the most dramatic responses.

place, unless by a few percent" (*22*, p. 133).

In light of these observations, the postulate that CBF is regulated by and for the sake of metabolic rate must also be reconsidered. The disproportionate increase in CBF that accompanies physiological neural activation causes P_{O_2} and *p*H to rise and P_{CO_2} to fall, rather than the reverse (*2*), arguing strongly against glucose oxidation as a regulator of CBF under physiological conditions. Paulson and Newman, however, have proposed a mechanism independent of metabolic rate by which physiological changes in neural activity may regulate regional changes in CBF (*23*): K^+ released by neural firing is taken into astroglial processes surrounding the neuron, siphoned through soma, and released from processes (end-feet) abutting the capillary, which is highly sensitive to K^+ concentration.

In conclusion, traditional concepts of the dynamic regulation of cerebral metabolism and blood flow must be reconsidered. Although resting energy needs are supported by glucose oxidation, transient increases in neural activity preferentially induce glycolysis and glycogen formation. This result implies that the acute energy expenditures of neural activity are far less than has been inferred from the large increases in glucose uptake and the high (4.1:1) resting-state O_2:glucose molar ratio. Finally, blood flow increases during neural activity are regulated by a mechanism, and serve a need, other than oxidative metabolism.

REFERENCES AND NOTES

1. B. Siesjo, *Brain Energy Metabolism* (Wiley, New York, 1978), pp. 101–110.
2. P. T. Fox and M. E. Raichle, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1140 (1986).
3. All subjects were normal volunteers between the ages of 19 and 26 years. This study was approved by the Radioactive Drug Research Committee (Food and Drug Administration) of Washington University. Informed consent was obtained by using forms and procedures approved by the Human Studies Committee of Washington University.
4. P. Herscovitch, J. Markham, M. E. Raichle, *J. Nucl. Med.* **24**, 782 (1983); M. E. Raichle, W. R. W. Martin, P. Herscovitch, M. A. Mintun, J. Markham, *ibid.*, p. 790; P. Herscovitch, M. E. Raichle, M. R. Kilborn, M. E. Welch, *J. Cereb. Blood Flow Metab.* 7, 527 (1987); M. A. Mintun, M. E. Raichle, W. R. W. Martin, P. Herscovitch, *J. Nucl. Med.* **25**, 177 (1984); T. O. Videen, J. S. Perlmutter, P. Herscovitch, M. E. Raichle, *J. Cereb. Blood Flow Metab.* 7, 513 (1987); W. R. W. Martin, W. J. Powers, M. E. Raichle, *ibid.*, p. 421. The CBF was measured with a bolus intravenous injection of ^{15}O-labeled water with a 40-s emission scan initiated as the tracer entered the brain. Units of CBF were milliliters per minute per 100 g of brain tissue. $CMRO_2$ was measured with a single-breath inhalation of ^{15}O-labeled molecular O_2, also with a 40-s emission scan initiated on tracer entry to brain. Units of $CMRO_2$ were micromoles per minute per 100 g of brain tissue. Measurements of $CMRO_2$ were corrected for nonmetabolized, intravascular oxygen with a cerebral blood volume (CBV) scan. CBV was measured with a single-breath inhalation of ^{15}O-labeled carbon monoxide with a 300-s equilibrium emission scan.
5. M. Rievich *et al.*, *J. Cereb. Blood Flow Metab.* **5**, 179 (1985). CMRglu was measured with an intravenous bolus of ^{18}F-labeled 2-fluoro-2-deoxy-D-glucose (^{18}F-DG) with a 10-min emission scan initiated 45 min after tracer administration. Units of CMRglu were micromoles per minute per 100 g of brain tissue. Standard values were used for rate constants and the lumped constant. True arterial sampling, rather than arterialized venous, was used. The second injection of ^{18}F-DG was given 1 hour after completion of the first CMRglu scan and was preceded by a background scan that was corrected for isotope decay and subtracted from the second ^{18}F-DG image before conversion to physiological units of CMRglu.
6. P. T. Fox *et al.*, *J. Neurosci.* 7, 913 (1987). Focal, physiological neural activation was induced by patterned visual stimulation. The resting state was eyes-closed rest. During the stimulated-state measurements, subjects maintained fixation on a central cross hair while viewing an annular checkerboard extending from 1.5° to 15.5° of eccentricity. Colors were red on black and reversed at 10 Hz to maximize the CBF response. P. T. Fox and M. E. Raichle, *J. Neurophysiol.* **51**, 1109 (1984).
7. J. S. Perlmutter, P. Herscovitch, W. P. Powers, P. T. Fox, M. E. Raichle, *J. Cereb. Blood Flow Metab.* **5**, 476 (1985). Forty-eight (24 per hemisphere) discrete brain regions were stereotactically identified in each subject. These regions were used for computation of whole-brain mean values and for multiregional correlation analysis.
8. J. C. Baron *et al.*, *J. Nucl. Med.* **23**, 391 (1982).
9. Although the $CMRO_2$ changes reported here did not reach statistical significance (Table 1), our cumulative data did.
10. W. Penfield, *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **18**, 605 (1937); *J. Neurosurg.* **35**, 124 (1971).
11. F. Plum, J. B. Posner, B. Troy, *Arch. Neurol.* **18**, 1 (1968); T. G. Bolwig, Z. E. Regon, O. J. Rafaelsen, N. A. Lassen, *ibid.* **28**, 334 (1973).
12. R. Cooper, H. J. Crow, W. G. Walter, A. L. Winter, *Brain Res.* **3**, 174 (1966).
13. M. Reivich *et al.*, *J. Neurochem.* **15**, 301 (1968); R. E. Townsend, P. M. Prinz, W. D. Obrist, *J. Appl. Physiol.* **35**, 620 (1973).
14. W.-D. Heiss, G. Pawlik, K. Herholz, K. Wienbard, *Brain Res.* **327**, 362 (1985).
15. J. S. Meyer and M. Toyoda, in *Cerebral Circulation and Stroke*, K. J. Zulch, Ed. (Springer-Verlag, New York, 1971), pp. 156–163; T. V. Santiago, E. Guerra, J. A. Neubauer, N. H. Edelman, *J. Clin. Invest.* **73**, 497 (1984).
16. M. D. Ginsberg *et al.*, *Ann. Neurol.* **23**, 152 (1988).
17. K.-A. Hossman and F. Linn, *J. Cereb. Blood Flow Metab.* 7, S297 (1987).
18. J. W. Prichard, O. A. C. Petroff, T. Ogino, R. Shulman, *Ann. N.Y. Acad. Sci.* **508**, 54 (1987).
19. R. A. Swanson, F. R. Sharp, S. M. Sagar, *Soc. Neurosci. Abstr.* **13**, 649 (1987).
20. Magistretti *et al.* have generated considerable evidence that cortical glycogen is extremely biochemically active and is regulated by K^+ and other substances to support local neural activity. P. R. Hof, E. Pascale, P. J. Magistretti, *J. Neurosci.* **8**, 1922 (1988); P. J. Magistretti, P. R. Hof, J.-L. Martin *ibid.* **6**, 2558 (1986).
21. O. Creutzfeldt, in *Brain Work: The Coupling of Function, Metabolism, and Blood Flow in the Brain*, D. H. Ingvar and N. A. Lassen, Eds. (Alfred Benzon Symposium VIII, Munksgaard, Copenhagen, 1975), pp. 22–47.
22. C. Van den Berg, in *Energetics and Human Information Processing*, G. R. J. Hockey, A. W. K. Gaillard, M. G. H. Coles, Eds. (Nijhoff, Boston, 1986), pp. 131–135.
23. O. B. Paulson and E. A. Newman, *Science* **237**, 896 (1987).

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Fertilization Events Induced by Neurotransmitters After Injection of mRNA in *Xenopus* Eggs

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Fertilization initiates in the egg a dramatic increase in intracellular calcium that opens ion channels and causes exocytosis. To explore the possibility that these events might involve a receptor-mediated pathway, receptors for serotonin or acetylcholine (M1 muscarinic) were expressed in the *Xenopus* egg; serotonin or acetylcholine then could initiate a series of responses similar to those normally initiated by sperm. Thus, there may be an endogenous receptor in the egg membrane that is activated by sperm, and the serotonin or M1 muscarinic receptor may replace the sperm receptor in this pathway.

THE ACTIVATION OF THE EGG BY THE sperm is similar to interactions of neurotransmitters and hormones with membrane receptors; in particular, fertilization appears to activate a guanine nucleotide binding (G) protein leading to inositol 1,4,5-trisphosphate (IP_3) production and Ca^{2+} release (*1, 2*). Release of Ca^{2+} then causes diverse responses in the egg, including ion channel opening and cortical granule exocytosis (*3*). Although a receptor that mediates the binding of sperm to the egg's extracellular coat has been identified (*4*), it is not known whether there is a receptor in the plasma membrane of the egg that mediates the activation process.

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