

formulas for pre-term infants with low body weight, to achieve the maximum growth rate and food efficiency.

Every new finding generates questions. For example, why should dietary proteins have different effects on protein turnover? Is this finding unique to whey protein and casein, or can it be applied to other dietary proteins? Are there striking differences between proteins from animal and vegetable origin? What is the site of postprandial protein deposition? Does the effect on protein metabolism differ between lean and obese people? But perhaps the first step is to determine the extent to which the effect of the amino-acid absorption rate on protein metabolism is reproducible when proteins are given under more physiological circumstances — as part of a typical, mixed meal. □

Gema Frühbeck is at the MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH, UK.

e-mail: Gema.Fruhbeck@mrc-dunn.cam.ac.uk

- Boirie, Y. *et al.* *Proc. Natl. Acad. Sci. USA* **94**, 14930–14935 (1997).
- Garlick, P. J., McNurlan, M. A. & Ballmer, P. E. *Diabetes Care* **14**, 1189–1198 (1991).
- Pacy, P. J., Price, G. M., Halliday, D., Quevedo, M. R. & Millward, D. J. *Clin. Sci.* **86**, 103–118 (1994).
- Waterlow, J. C. *Annu. Rev. Nutr.* **15**, 57–92 (1995).
- Nissen, S. & Haymond, M. W. *Am. J. Physiol.* **250**, E695–E701 (1986).
- Young, V. R., Gucalp, C. R., Rand, W. M., Matthews, D. E. & Bier, D. M. *Hum. Nutr. Clin. Nutr.* **41**, 1–18 (1987).
- Mahé, S. *et al.* *Am. J. Clin. Nutr.* **63**, 546–552 (1996).
- Giordano, M., Castellino, P. & DeFronzo, R. A. *Diabetes* **45**, 393–399 (1996).
- Daniel, H., Vohwinkel, M. & Rehner, G. *J. Nutr.* **120**, 252–257 (1990).
- Garlick, P. J. & Reeds, P. J. in *Human Nutrition and Dietetics* (eds Garrow, J. S. & James, W. P. T.) 56–76 (Churchill Livingstone, Edinburgh, 1993).

## Neurobiology

# Homeostasis or synaptic plasticity?

Yves Frégnac

Almost 50 years ago, Hebb<sup>1</sup> proposed that during development, learning and perception, correlated activity induces long-lasting strengthening in synaptic transmission. Remarkably, however, neurobiologists inspired by Hebb's principle have sought only one side of the regulatory process — that is, synapse-specific changes in synaptic strengths. They have rarely thought about the compensatory mechanisms that regulate the total synaptic strength of a neuron (reviewed in ref. 2). But on page 892 of this issue, Turrigiano *et al.*<sup>3</sup> describe stabilizing mechanisms that may represent a more general form of activity-dependent regulation of synaptic transmission. The changes in postsynaptic sensitivity that they have found might be seen as a demonstration of basic homeostasis, designed to return the integrative function of the cell to within a reference working range.

Five homeostatic processes related to synaptic integration are generally recognized. The simplest form regulates the efficacy of transmission around a mean synaptic gain or between two boundary values. When evaluated on a longer timescale, the fast, input-dependent regulation of synaptic transmission (recently described in cortical networks<sup>4,5</sup>) results in an averaged synaptic efficacy that is roughly constant in the face of rapid changes in the probability of transmitter release<sup>6</sup>. Furthermore, the probability of inducing potentiation, depression or depotentiation depends on the previous stimulation history of the network ('metaplasticity' in ref. 7) and on the initial state of the synapse.

The second form of homeostasis — predicted in many models of learning — acts more globally, at the neuronal level<sup>8</sup>. It assumes that the sum of all the synaptic

weights of synapses impinging on the cell (or of their square values) remains constant. A third form of homeostasis limits the anatomical divergence of growing axons, corresponding to a conserved sum of the synaptic weights of all contacts made in the network by the same parent axon<sup>9</sup>. The fourth form of homeostasis, which is relevant to the findings of Turrigiano *et al.*<sup>3</sup>, concerns the capacity of a cell to maintain a similar output (for example, its firing level), despite strong alterations in the activity pattern of the network (reviewed in ref. 10). This regulatory process involves activity-dependent tuning of postsynaptic sensitivity, and examples so far come from the regulation of intrinsic conductances<sup>11</sup>. A last form of homeostasis concerns possible changes in the site at which an impulse is initiated and the direction in which it is propagated in the dendrite<sup>12</sup>.

Turrigiano *et al.*<sup>3</sup> have observed up- and downregulation of the total postsynaptic strength, which does not operate in a synapse-specific manner. They compared AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) miniature excitatory postsynaptic current (mEPSC) amplitudes in 4–5-day cultures of visual cortex, bathed for tens of hours either with tetrodotoxin (to block spiking activity) or with a GABA<sub>A</sub> antagonist, bicuculline (to increase firing rates). They found that periods of artificially increased activity lead to a decrease in synaptic gain, as predicted from competitive learning. In contrast, after artificially maintained periods of decreased activity, they observed the reverse effect — that is, an increase in synaptic gain.

Although the amplitude of mEPSCs was altered, neither their frequency nor their kinetics varied as a result of the past activity of the network. Thus, the observed effect is

due to changes in postsynaptic AMPA-receptor sensitivity, affecting spike-induced EPSCs as well as miniature synaptic events. This process, described in cultures of pyramidal neurons of rat visual cortex, differs from the supersensitivity that has been found at the neuromuscular junction. Here, the enhancement is extrajunctional, and no change in the amplitude of the miniature synaptic events is observed.

But interpretation of the results may be more complex than it seems. First, these observations have been made in a cultured system only. Certain forms of plasticity that are found only in cultures may signal to the rest of the network a failure of a local synaptic homeostatic regulation. Depression signals spread to other synapses from the site at which the depression is induced, possibly because the general state of the cultured network is too far from equilibrium<sup>13</sup>. Second, these results need to be validated relative to other processes that are also known to occur. For example, the development of multiple synaptic contacts is characteristic, and an inverse relationship between the number of synapses and quantal amplitude is found, independent of time<sup>14</sup>.

Another consideration is that after treatment with tetrodotoxin or bicuculline, morphological and electrophysiological changes have been found which depend on the activation of NMDA (*N*-methyl-D-aspartate) receptors. Although Turrigiano *et al.* used the NMDA-receptor antagonist AP5 (*D*(-)-amino-7-phosphonovaleric acid), this does not exclude the involvement of NMDA in inducing the effects. A more probing test would have been to associate AP5 treatment with the blockade of AMPA receptors rather than applying it alone, which here does not change the network's activity. Finally, the effect might be specific to the culture or cell type. In other cultures, opposite effects that support a presynaptic mechanism have been reported<sup>15</sup>.

These remarks should not diminish the importance of the new findings<sup>3</sup>. The form of synaptic plasticity described by Turrigiano *et al.* operates very differently from long-term potentiation and long-term depression. Moreover, because it is bidirectional, it may have interesting computational properties. Indeed, the scaling process may have two facets. The homeostasis side comes from the fact that multiplicative scaling of synaptic strengths will preserve relative differences between inputs, while maintaining the output of a given cell roughly constant. But at the same time, the activity requirements for inducing synaptic changes in the network will also be modified. When inputs are scaled up or down, co-activation of a smaller or larger set of afferents will be required to depolarize the postsynaptic cell sufficiently, or to trigger an increase in calcium levels to induce plasticity.

This view is reminiscent of the hypothesis

of a floating threshold in postsynaptic plasticity, proposed for the visual cortex on purely theoretical grounds<sup>16</sup> and later worked out in more physiological terms<sup>17</sup>. The hypothesis holds that the postsynaptic plasticity threshold controlling the transition from long-term depression to long-term potentiation depends on the mean past activity of the cell. The plasticity threshold becomes easier or more difficult to reach by the cell's internal state, depending on whether the mean past neuronal activity — reflecting that of the whole network — has been reduced or increased, respectively. The mechanism described by Turrigiano *et al.* could play a similar role by scaling synaptic gain as a function of past activity. Linking the homeostasis of postsynaptic strength to functional plasticity remains the next challenge — and this will mean leaving the culture model for the more uncertain living brain. □

Yves Frégnac is in the Equipe Cognisciences, Institut Alfred Fessard, CNRS, 91198 Gif sur Yvette, France. e-mail: Yves.Fregnac@iaf.cnrs.gif.fr

1. Hebb, D. O. *The Organization of Behavior: a Neurophysiological Theory* (Wiley, New York, 1949).
2. Frégnac, Y. in *Handbook of Brain Theory and Neural Networks* (ed. Arbib, M.) 459–464 (MIT Press, 1995).
3. Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C. & Nelson, S. B. *Nature* **391**, 892–896 (1998).
4. Markram, H. & Tsodyks, M. *Nature* **382**, 807–810 (1996).
5. Abbott, L. F., Varela, J. A., Sen, K. & Nelson, S. B. *Science* **275**, 220–224 (1997).
6. O'Donovan, M. J. & Rinzel, J. *Trends Neurosci.* **20**, 431–433 (1997).
7. Abraham, W. C. & Bear, M. F. *Trends Neurosci.* **19**, 126–130 (1996).
8. Glanzman, D. L., Kandel, E. R. & Schacher, S. *Neuron* **7**, 903–913 (1991).
9. Willshaw, D. G. & Von der Malsburg, C. *Proc. R. Soc. Lond. B.* **194**, 431–445 (1976).
10. Miller, K. D. *Neuron* **17**, 371–374 (1996).
11. Turrigiano, G., Abbott, L. F. & Marder, E. *Science* **264**, 974–977 (1994).
12. Chen, W. R., Midtgaard, J. & Shepherd, G. M. *Science* **278**, 463–467 (1997).
13. Fitzsimonds, R. M., Song, H. J. & Poo, M. M. *Nature* **388**, 439–448 (1997).
14. Liu, G. & Tsien, R. W. *Nature* **375**, 404–408 (1995).
15. Tong, G., Malenka, R. C. & Nicoll, R. A. *Neuron* **16**, 1147–1157 (1996).
16. Bienenstock, E., Cooper, L. N. & Munro, P. J. *Neurosci.* **2**, 32–48 (1982).
17. Bear, M. F. *Neuron* **15**, 1–4 (1995).

Diabetes

## A signal for $\beta$ -cell failure

Joseph Avruch

Human type 2 diabetes is almost always accompanied by defects in both the responsiveness to insulin (which is commonly called insulin resistance) and the secretion of insulin. The result is hyperglycaemia — an excess of glucose in the bloodstream. Severe insulin resistance alone does not usually cause sustained hyperglycaemia, because the pancreatic  $\beta$ -cells can secrete much more insulin than is normally required. Moreover, in the face of insulin resistance, the  $\beta$ -cells have a substantial compensatory reserve. Although insulin resistance increases with the development of obesity and physical deconditioning, hyperglycaemia results mainly when the compensatory overproduction of insulin fails<sup>1</sup>.

On page 900 of this issue, Withers *et al.*<sup>2</sup> describe the phenotype of mice that lack both of the alleles encoding the insulin-receptor substrate protein-2, IRS-2. These mice develop a syndrome that mimics the common form of human type 2 diabetes. As expected, the IRS-2 knockout mice are considerably insulin resistant. But, in contrast to other murine models with severe insulin resistance, which show a marked increase in  $\beta$ -cell mass, the IRS-2 knockout mice have a smaller mass of  $\beta$ -cells than wild-type mice. Thus, Withers *et al.* have not only verified the importance of IRS-2 in insulin action, but they have identified IRS-2 as one of the molecular determinants of  $\beta$ -cell compensation.

The IRS proteins were first detected as polypeptides (relative molecular mass,  $M_r$ , 160,000–185,000) that underwent rapid

tyrosine phosphorylation in response to insulin and insulin-like growth factor-1 (IGF-1), but not to other growth factors that act through receptor tyrosine kinases<sup>3</sup>. The IRS-1 protein contains an amino-terminal pleckstrin homology domain, followed by a phosphotyrosine-binding domain. Together, these enable binding to the activated insulin/IGF-1 receptor. The IRS proteins also have long carboxy-terminal tails that contain 8–18 potential tyrosine phosphorylation sites, many of which are in a YXXM motif — a preferred sequence for the insulin/IGF-1 receptor kinases. Once tyrosine phosphorylated (Fig. 1), IRS-1 binds proteins that contain SH2 domains, and, among this array, the phosphatidylinositol-3-OH kinase is probably the most important to the insulin regulation of energy metabolism.

Four IRS isoforms are currently known. The IRS-2 protein ( $M_r$ , 180,000) was first found in myeloid progenitor cells. IRS-3 ( $M_r$ , 60,000) is an adipocyte-specific IRS isoform<sup>4</sup>, and IRS-4 ( $M_r$ , 160,000) is a distinct homologue<sup>5</sup>. Because tyrosine phosphorylation of IRS-2 is promoted by interleukin-4 (as well as by insulin and IGF-1), the role of IRS proteins in the actions of other ligands whose receptors signal through Janus-family non-receptor tyrosine kinases was examined. The interferons, and many members of the interleukin-2- and interleukin-6-receptor families (including, for example, growth hormone), are now known to stimulate tyrosine phosphorylation of the IRS proteins<sup>3</sup>.

What functions the IRS proteins provide, that are not already supplied by the docking sites found on the receptor and non-receptor tyrosine kinases themselves, is not yet known.

So what happens when the IRS proteins are knocked out? *IRS-1* knockout mice show a surprisingly modest phenotype<sup>6,7</sup>. They have normal development at birth, but they are only 50–60% the weight of wild-type mice and remain comparatively small through life. Despite being clearly insulin resistant, these mice rarely develop fasting hyperglycaemia. Although insulin signal transduction is greatly reduced in skeletal muscle, it is maintained in the liver, and  $\beta$ -cell compensation is robust.

With such a relatively weak phenotype in homozygous knockout mice, one is inclined to discount the importance of heterozygous point mutations in the human *IRS-1* gene. But functionally significant polymorphisms have been identified in the *IRS-1* gene, and these are more frequent in patients with type 2 diabetes. Moreover, heterozygous deficiency of the *IRS-1* and insulin-receptor genes in mice — although each alone is a silent event — together produce a strong diabetic phenotype<sup>8</sup>. The genetic basis for insulin resistance may, therefore, derive from the multiplicative effects of common polymorphisms at several steps in the insulin signalling pathway, each causing only a modest functional impairment.

In *IRS-1* knockout mice, expression of *IRS-2* is upregulated in skeletal muscle, suggesting that *IRS-2* may compensate, in part, for the *IRS-1* deficiency. Withers *et al.*<sup>2</sup> now establish *IRS-2* not only as an important player in insulin signalling, but also as a candidate diabetes gene in man<sup>9</sup>. The unexpected element in the *IRS-2* knockout phenotype is the failure of robust  $\beta$ -cell compensation. The compensatory increase in  $\beta$ -cell mass that occurs in insulin-resistant states reflects some combination of islet-cell hypertrophy, replication, neogenesis by differentiation from ductal precursors, and diminished apoptosis<sup>10</sup>.

How does a deficiency of *IRS-2* curtail  $\beta$ -cell compensation? Withers *et al.* show that *IRS-2* colocalizes with insulin in pancreatic islets, and that *IRS-2* is expressed in ductal precursor cells. The  $\beta$ -cells also express *IRS-1*, insulin receptors and IGF-1 receptors. The signalling elements that are controlled by *IRS-2* (such as phosphatidylinositol-3-OH kinase and Ras) are crucial regulators of mitogenesis, cellular differentiation and apoptosis. Identification of the downstream targets of these signals in the  $\beta$ -cell and its progenitors will be crucial to the understanding and manipulation of  $\beta$ -cell fate.

Pancreatic  $\beta$ -cells continue to replicate throughout a rodent's life, balanced by cell death, and gene knockouts of several transcription factors have provided an outline of