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Food restriction enhances brain plasticity in adult rats

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Contents

INTRODUCTION

The term plasticity has been used in brain science for well over a century and it refers to the changes that occur in the organization of the brain as a result of experience (for an historical review on the term plasticity see Berlucchi and Buchtel 2009). The word experience accounts for various form of behavioural modifiability, either short or long-lasting, including maturation, adaptation to a mutable environment, learning and compensatory adjustments in response to functional losses during ageing or brain damage.

Every single behavioural task performed by the mature nervous system, from the perception of the sensory input and the control of motor output to cognitive functions such as learning and memory, depends on the definite interconnections of many millions of neurons. Such a defined wiring is largely controlled by molecular programs, but the precise matching of presynaptic neurons to postsynaptic targets widely depends on neural activity, either spontaneous or evoked by sensory-driven experience (Goodman and Shatz 1993; Katz and Shatz 1996). There are periods in early postnatal life, the so called critical periods (CPs), during which neural circuits display a heightened sensitivity to certain environmental stimuli and are shaped by natural sensory experience.

These periods of high plasticity have been found in all species tested so far, from humans to Drosophila (Barth et al., 1997) and affect the development of the primary sensory systems (Hensch, 2004; Berardi et al. 2000).

One crucial question about CP plasticity is why experience is so effective in eliciting great structural and functional changes only during a restricted period of postnatal life. What determines the beginning of the CP? What makes the CP end?

Recently, several studies in the visual system shed a new light on this field in-

dicating that the adult brain is not hard-wired with fixed and immutable neuronal circuits. On the contrary, it keeps a certain degree of plasticity in response to proper stimuli (for a review see Spolidoro et al., 2009). Given these important results, studying the mechanisms that reduce plasticity in adulthood has begun even more challenging considering the great impact that it could have in clinical neurology. Furthermore, the possibility to influence the brain by simply changing some aspects of the individual lifestyle has recently emerged (Sale et al, 2007a). This opened a new fascinating field in the therapeutic neuroscience indicating that non-invasive treatments could exert significant and helpful benefits both at the level of rehabilitation from injuries and amelioration of everyday life conditions.

Nutrition is a fundamental component of our life. The quality and quantity of food consumed by each individual is critical for the development and maintenance of its general physical structure. A great number of studies have identified several specific dietary components that are essential for the correct development of the central nervous system (Mattson et al., 2003). Interestingly, it has been demonstrated that simply reducing the caloric intake can lead to an extension of the mean and maximum lifespan in a wide range of organisms including yeasts, worms, fruit flies, rodents and monkeys (Masoro, 2001). This increase in longevity is often accompanied by an effective counteraction of the aging process both at the level of body healthness with reduced incidence of age-related cardiomyopathy, diabetes, hypertension and neoplastic processes (Masoro, 1993; Mattson et al, 2002) and at the level of brain function with a delay in the onset of learning and memory decline (Idrobo et al., 1987; Stewart et al, 1989) and suppression of LTP deficits in the hippocampus (Hori et al., 1992; Eckles-Smith et al, 2000).

The mechanisms by which dietary restriction leads to increased maximal and average lifespan and to decrease of age-related pathologies remain unclear. One fascinating hypothesis is that the reduction in caloric intake could be accompanied or could even exert its effects by influencing brain plasticity. The main purpose of my study was to test this possibility and to give, for the first time, evidence that food restriction (FR) increases brain plasticity in adult, not aged, healthy animals.

In the following chapters I will start describing the main knowledge about the

mammals visual system, the paradigmatic model I used to study plasticity in the CNS. The second chapter will deal with the reinstatement of plasticity to the adult and some of the treatments which have been used in the last years in order to achieve this goal. In the third chapter the general benefits of FR will be analyzed in depth and a discussion of the molecular mechanisms involved in the longevity effects of caloric restriction will be provided. Finally I will explain the details of my work, presenting my results an examining their significance and implications.

The visual system in mammals

Phototransduction and information processing in the retina

Visual perception occurs in two stages. Light entering the cornea is projected on the back of the eye where it is converted into an electrical signal by a specialized sensory organ, the retina. These signals are then sent through the optic nerve to higher centres in the brain for further processing.

Anatomical organization of the retina. The retina bears careful examination for several reasons. First, it is a useful model system for understanding sensory transduction. Indeed, light is converted in electrical signals by specialized retinal neurons called photoreceptors. Second, unlike other sensory structures, such as the cochlea or somatic receptors in the skin, the retina is not a peripheral organ but part of the central nervous system. Compared to other brain regions, however, the retina is relatively simple. It contains only five major classes of neurons linked in an intricate pattern of connections, but with an orderly, layered anatomical arrangement. This combination of physiological diversity and relatively simple structural organization makes the retina useful for understanding how information is processed by complex neural circuits in the brain.

The eye is foremost an optical device designed to focus the visual image on the retina with minimal optical distortion. Light is focused by the cornea and the lens, then traverses the vitreous humour that fills the eye cavity before being absorbed by the photoreceptor cells. The retina is apposed to the pigment epithelium that lines the back of the eye. Cells in the pigment epithelium are packed with the black pigment melanin, which absorbs any light not captured by the retina, thereby preventing it from being reflected back to the retina (which would degrade the visual image).

Pigment epithelial cells also assist photoreceptors with important aspects of their metabolism. For this reason, photoreceptors directly contact the pigment epithelium, while the other retinal cells are closer to the lens.

In one region of the retina, the fovea, the cell bodies of the proximal retinal neurons have been shifted to the side, enabling the photoreceptors to receive the visual image in its least distorted form. Nasal to the fovea is the optic disc where the optic nerve fibers leave the retina. This region has no photoreceptors and therefore creates a blind spot in the visual field.

Photoreceptors. The human retina contains two types of photoreceptors, rods and cones. Cones are responsible for day vision while rods mediate night vision, they function in the dim light (for example at dusk or night) when most stimuli are too weak to excite the cone system.

The brain obtains information about color by comparing the responses of three types of cones, each with a visual pigment that is more senisitive to a different part of the spectrum. In contrast, there is only one rod pigment so that all rods respond in the same way to different wavelenghts. Rod vision is therefore achromatic.

Rods and cones have similar functional regions: 1) a region specialized for phototransduction, called the outer segment (because it is located at the outer or distal surface of the retina); 2) a region containing the cell's nucleus and most of its biosynthetic machinery, called the inner segment; 3) a synaptic terminal that makes synaptic contact with the photoreceptor's target cells.

The outer segments of rods and cones are effective light-catchers because they are densely packed with light-absorbing visual pigments.

The absorption of light by visual pigments in rods and cones triggers a cascade of events that eventually leads to a change in ionic fluxes across the plasma membrane of these cells and a consequent change in membrane potential. A key intermedite in this cascade is the cyclic nucleotide 3'-5' cyclic guanosine monophosphate, or cGMP.

Phototransduction. Phototransduction occurs in three stages: 1) light activates visual pigments; 2) these activated molecules cause the stimulation of cGMP phos-

phodiesterase, an enzyme that reduces the cytoplasmic concentration of cGMP; 3) the reduction in cGMP concentration closes the cGMP-gated channels, thus hyperpolarizing the photoreceptor.

The change in the photoreceptor's membrane potential during illumination is determined both by the change in the current that flows through the cGMP-gated channels and by a current flowing across the photoreceptor membrane through K^+-selective, nongated (leakage) channels that are like those of other neurons. In darkness the high resting levels of cGMP in the cell keep the cGMP-gated channels open, allowing an inward current of about 50 pA, largely carried by Na^+ ions, to flow into the cell. This steady inward current, called the dark current, maintains the photoreceptor membrane potential at around 40 mV.

When light reduces cGMP and the cGMP-gated channels close, the inward Na^+ current that flows through these channels is reduced, thus hyperpolarizing the cell.

Retinal interneurons and ganglion cells. The output neurons of the retina are the ganglion cells. Their axons form the optic nerve, which project to the lateral geniculate nucleus (LGN) and to the superior colliculus as well as to brain stem nuclei. Unlike photoreceptors, which respond to light with graded changes in membrane potential, ganglion cells transmit information as trains of action potentials. Sandwiched between the photoreceptors and the ganglion cells are three classes of interneurons: bipolar, horizontal and amacrine cells. These cells transmit signals from the photoreceptors to the ganglion cells. They also combine signals from several photoreceptors. Thus, the electrical responses evoked in ganglion cells depend critically on the precise pattern of the light that stimulates the retina and how this pattern changes with time.

Ganglion cells are never silent, even in the dark, but light modulates their spontaneous activity. Each ganglion cell responds to light directed to a specific area of the retina. This area is called the receptice field of the cell. The receptive field of a ganglion cell (or any other cell in the visual pathway) is that area of the retina where stimulation of photoreceptors causes either an increase or decrease of the ganglion cell's firing rate. Ganglion cell receptive fields have three important features:

- They are circular and they vary in size across the retina. In the foveal region of

the primate retina, where visual acuity is greatest, the receptive fields are small, at the periphery of the retina, where acuity is lower, the fields are larger;

- They have a center and an antagonistic surround;
- Ganglion cells process visual information in two parallel pathways. Two classes of ganglion cells can be distinguished by their response to a small spot of light applied to the center of their receptive field. On-center ganglion cells fire few action potentials in darkness, and light directed to the center of their receptive field increases their firing rate. Light applied to the surround inhibits the effect produced by illumination of the center; the most effective inhibitory stimulus is a ring of light on the entire surround. Off-center ganglion cells are inhibited by light applied to the center of their receptive field. Light excites an off-center ganglion cell when it is directed to the surround of the receptive field.

Each type of interneuron in the retina (horizontal, bipolar and amacrine cells) palys a specific role in shaping photoreceptor signals as they are transmitted through the retina. The bipolar cells represent the most direct pathway between the receptors and ganglion cells.

Visual information is transferred from cones to ganglion cells along two types of pathways in the retina. Cones in the center of a ganglion cell's receptive field make direct synaptic contact with bipolar cells that in turn directly contact the ganglion cells (direct or vertical pathway). Signals from cones in the surround of the ganglion cell's receptive field are conveyed to the ganglion cell by means of horizontal and amacrine cells (lateral pathways). Horizontal cells tranfer information from distant cones to nearby bipolar cells. Some type of amacrine cells tranfer information from distant bipolar cells to the ganglion cells.

Bipolar cells are the key interneuron in the retina. They have complex receptive field properties like those of ganglion cells.

The cones in the receptive field center of a bipolar cell appear to be directly connected to the bipolar cell. On-center bipolar cells depolarize, while off-center bipolar cells hyperpolarize when light stimulates cones in the center of their receptive field. In

contrast, the inputs of cones in the bipolar cell's surround are relayed by horizontal cells, which can respond to inputs from distant sources because they have large dendritic trees and because they are electrically connected to other horizontal cells by gap junctions. When light stimulates cones in a bipolar cell's surround, it produces the opposite response to that evoked by illumination of cones in the center.

The cones release a single neurotransmitter, thought to be the glutamate, which has opposite actions on the two classes of bipolar cells. On-center bipolar cells are inhibited and off-center bipolar cells are excited (recall that the cone is depolarized in the dark).

Glutamate produces different responses in the two classes of bipolar cells by gating different ion channels.

The receptive field properties of a ganglion cell largely reflect those of the bipolar cells connected to it because each type of bipolar cell (on-center or off-center) makes excitatory synaptic connections with the corresponding type of ganglion cell.

Central visual pathways

The regions of the visual field are defined with respect to the two retinas. The regions of the retina are named with reference to the midline: the nasal hemiretina lies medial to the fovea, and the temporal hemiretina is lateral to the fovea. The visual field is the view seen by the two eyes without movement of the head. If the foveas of both eyes are fixed on a single point in space it is possible to define a left and a right half of the visual field. Light originating in the central region of the visual field enters both eyes, this area is called the binocular zone. In either half of the visual field there is also a monocular zone: light from the temporal portion of the hemifield projects only on the nasal hemiretina of the eye on the same side because the nose blocks this light from reaching the eye on the opposite side.

The axons of all the retinal ganglion cells stream toward the optic disc, where they become myelinated and together form the optic nerve. The optic nerves from each eye join at the optic chiasm. There, fibers from each eye destined for one or the other side of the brain are sorted out. Retinal fibers fro both eyes then enter each optic tract, which projects to three subcortical targets. Of the three subcortical regions receiving

direct input from the retina, only one, the LGN, processes visual information that ultimately results in visual perception. The pretectal area of the midbrain uses inputs from the retina to produce pupillary reflexes, whereas the superior colliculus uses its input to generate eye movements.

Lateral geniculate nucleus. The majority of retinal axons terminate in the LGN. Axons from the retina project through the optic chiasm, where the fibers from the nasal half of each retina cross the opposite side of the brain. The axons from ganglion cells in the temporal hemiretina do not cross.

Fibers from the right half of each retina (the nasal hemiretina of the left eye and the temporal hemiretina of the right eye) project in the right optic tract to the right LGN. Similarly, fibers from the left hemiretina of each eye project in the left optic tract to the left LGN.

Ganglion cells in the retina project in an orderly manner to points in the LGN, so that in each LGN there is a visuotopic representation of the contralateral half of the viasual field.

The LGN of primates contains six layers of cell bodies separated by intervening layers of axons and dendrites. The layers are numbered from 1 to 6, ventral to dorsal. An individual layer in the nucleus receives input from one eye only: fibers from the contralateral nasal hemiretina contact layers 1, 4, and 6; fibers from the ipsilateral temporal hemiretina contact layers 2, 3 and 5.

Receptive fields of neurons in the LGN are the same as those found in the retina: small concentric fields either on or off-center. Like the retinal ganglion cells, neurons in the LGN respond best to small spots of light within their receptive field center. This similarity of the receptive properties of cells in the LGN and those of retinal ganglion cells derives in part from the fact that each geniculate neuron receives its main retinal input from only a very few ganglion cell axons with very little transformation of the incoming information.

As in the retina, the on and off-center pathways in the LGN are independent.

The primary visual cortex. The first relay point in visual processing where

receptive field properties change significantly is the primary visual cortex (V1). Like the LGN and superior colliculus the V1 in each cerebral hemisphere receives information exclusively from the contralateral half of the visual field.

The human visual cortex is about 2 mm thick and consists of six layers of cells (layers 1-6) between the pial surface and the underlying white matter. The cortex contains two basic classes of cells. Pyramidal cells are large and have long spiny dendrites; they are projection neurons whose axons project to other brain regions. Non pyramidal cells are small, stellate in shape, and have dendrites that are either spiny or smooth. They are local interneurons whose axons are confined to the primary visual cortex. The pyramidal and spiny stellate cells are excitatory and many use glutamate or aspartate as their transmitters; the smooth stellate cells are inhibitory and many contain γ-aminobutyric acid (GABA).

Once afferents from the LGN enter the V1, information flows systematically from one cortical layer to another, starting with the spiny stellate cells, which predominate in layer 4. These cells receive direct input from the LGN and project up to layers 2 and 3. Cells in layers 2 and 3 project down to pyramidal cells in layer 5, which then feed via axon collaterals to pyramidal cells in layer 6. The pyramidal cells of layer 6 complete the local excitatory circuit by sending axon collaterals to layer 4 to excite the inhibitory smooth stellate cells. These in turn contact and modulate the firing of the excitatory spiny stellate cells, completing an inhibitory feedback loop. Thus the spiny stellate cells distribute the input from the LGN to the cortex and the pyramidal cells feed axon collaterals upward and downward to integrate activity within the layers of V1.

Hubel, Wiesel and their collegues found that most cells above and below layer 4 respond only to stimuli that are substantially more complex than those that excite cells in the retina and LGN (Hubel and Wiesel 1959). The most astonishing finding was that small spots of light are completely ineffective in all layers of the visual cortex (except some regions called blobs). Cells in all regions except blobs respond only to stimuli that have linear properties, such as a line or bar. Hubel and Wiesel categorized the cells into two major groups, simple and complex, based on their reponses to linear stimuli. Simple cells resemble cells of the LGN, except that the on and off zones of

their receptive fields are rectangular with a specific axis of orientation. A stimulus with an orientation perpendicular or even oblique to the orientation of the cell's receptive field will be ineffective. Every axis of rotation is represented for every retinal position.

Hubel and Wiesel suggested that a rectilinear receptive field could be biult up from many circular fields in appropriate connections with stellate cells in the layer 4 of V1 (Hubel and Wiesel 1962). This idea has received direct support from studies by Stryker and collegues (1990) who found that the distribution of geniculate input on simple cortical cells predicts their axes of orientation.

The receptive fields of complex cells are usually larger than those of simple cells. These fields also have a critical axis of orientation, but the precise position of the stimulus within the receptive field is less crucial because there are no clearly defined on or off zones. Movement across the receptive field is a particularly effective stimulus for certain complex cells. Although some complex cells have direct connections with cells of layer 4, Hubel and Wiesel proposed that a significant input to complex cells comes from a family of simple cortical cells that have the same axis of orientation but slightly offset receptive field positions.

The convergent actions of cells in V1 are the initial steps in percetion. In its simplest form, this scheme suggests that each complex cell surveys the activity of a group of simple cells. The simple cells survey the activity of a group of geniculate cells, which themselves survey the activity of a group of retinal ganglion cells. The ganglion cells survey the activity of bipolar cells that survey a group of receptors. At each level, each cell has a greater capacity for abstraction than do the cells at the lower level.

At the lowest level of the system, the level of retinal ganglion and geniculate cells, neurons respond primarily to contrast. This elementary information is repatterned by the simple and complex cells of the cortex into rectangular fields with reltively precise line segments and boundaries. Thus, the stimulus requirements necessary to activate a cell become more precise at each level of the afferent system. In the retina and LGN stimulus position is important. In simple cells, in addition to position, the axis of orientation is important. In complex cells, whose receptive fields are larger, the axis of orentation is also important, but these cells have the ability to detect orientation over a wide range of positions. The V1 is organized in narrow colums, running from the pial

surface to the white matter. In each column there are simple cells with almost identical retinal positions and identical axes of orientation. For this reason these groupings are called orientation columns. Each orientation column also contains complex cells.

Detailed mapping of adjacent columns by Hubel and Wiesel, using tangential penetrations with microelectrodes, revealed a precise organization with an orderly shift in axis of orientation from one column to another. The systematic shifts in axis of orientation from one column to another is occasionally interrupted by blobs, peg-shaped regions of cells in layer 2 and 3 of V1. Cells in the blobs receive direct connections fom the LGN and are concerned with color.

In addition to columns devoted to axis orientation and blobs related to color, a third alternating system of columns is devoted to the left or the right eye. These ocular dominace columns are important for binocular interactions.

Hubel and Wiesel introduced the term hypercolumn to refer to a set of columns responsive to lines of all orientations from a particular region in space. A complete sequence of ocular dominance columns and orientation columns is repeated regularly and precisely over the surface of the V1. These columnar systems communicate with one another by means of horizontal connections that link cells within a layer. These connections integrate information over many millimeters of cortex. As a result, a cell can be influenced by stimuli outside its normal receptive field. Indeed, a cell's axis of orientation is not completely invariant but is dependent on the context on which the feature is embedded. The psychophysical principle, called the contextual effect, whereby we evaluate objects in the context in which we see them, is thought to be mediated through horizontal connections.

The Rat Visual System

The general concept of the visual system operating as a set of pathways working largely in parallel can be applied to all Mammals. However, results and interpretations of visual studies in the widely used cat and primate should not be uncritically extrapolated to the rat. There are some peculiarities of this species which are worth a note.

The rat, a nocturnal murid rodent, has sophisticated and effectively functioning visual system. Its laterally placed eyes provide it with a panoramic view but there is

a binocular overlap, estimated to be 40-60° in front of the animal.

Although rods are the predominant photoreceptors, cones are also present (about 0,85% of photoreceptors) and the retina is therefore capable of functioning in both scotopic and photopic conditions (Cicerone, 1976; Szél and Röhlich, 1992). Of the two types of cones identified, the majority (93%) contains a photopigment with a peak sensitivity at about 500-520 nm (Deegan and Jacobs, 1993). Unlike other dichromatic animals, but like some other nocturnal rodents, the rat appears to have photoreceptors sensitive to ultraviolet light.

As already said, visual information is processed in the retina and sent to different structures of the central nervous system through retinal ganglion cell axons, which represent the output of the retina. The largest retinal projection terminates in the dorsal LGN (dLGN). There is extensive literature on the small ipsilateral pathway in the rat that contributes to the binocular representation of the visual field in target nuclei. Only about 3% of all ganglion cells project ipsilaterally (Jeffrey, 1984). In albinos the ipsilateral projection is even smaller (about 1,5%) (Ahmed et al., 1996; Dreher et al., 1985; Lund, 1965). Despite the lack of lamination in dLGN of the rat, several authors have discerned regional variations in the distribution of cells of different sizes, in the composition of afferent axons, and in the patterns of retrograde degeneration after cortical lesions. Reese (1988) emphasized that the nucleus consists of an outer shell located caudodorsally and an inner core constituting the ventromedial part. Ipsilateral axons with fast and medium conduction velocities terminate medially, in the inner core (Fukuda et al., 1981; Hale, 1980; Hayhow et al., 1962; Reese, 1988; Reese and Cowey, 1983).

In primates and cats the talamic input representing the two eyes parcel cortical layer 4 into alternating, equal-size stripes, allowing to distinguish a clear anatomical organisation in columns of ocular dominance (Hubel and Wiesel, 1963; Shatz and Stryker, 1978). The major difference between the rat visual cortex and that of cat and other mammals is the lack of anatomical ocular dominance columns. However, Thurlow and Cooper (1988) found hints of a patchy organisation of ipsilateral and contralateral input in the visual cortex of the rat, using a functional mapping by means of deoxyglucose. This issue has been confirmed through electrophysiological techniques by Caleo

and collegues (1999).

The physiological properties of the visual cortical neurons of the rat are immature at the opening of the eyes (postnatal day 15, P15) and develop gradually during the first month of postnatal life.

Briefly, in the rat, visual cortical responses are sluggish and variable at P17, in particular they present habituation, that is the tendency of cell response to diminish after several stimulations. These condition has been found also in kittens. Cell responsiveness, evaluated in term of amplitude of cell discharge in response to an optimal stimulation, increases progressively from P19-23 to P30, when values are superimposable to adult values. Spontaneous discharge is almost absent at P17 and it increases to reach adult values at around P30.

Ocular dominance (OD) distribution does not change significantly through development. Indeed, the vast majority of visual cortical neurons are binocular and preferentially driven by the contralateral eye. The major component of age-dependent changes in OD distribution is the increase of monocular, contralaterally driven cells. Furthermore, there is a clear decrease in the number of unresponsive cells, which is pretty high in very young animals but rapidly declines, reaching adult values at P30.

Properties like selectivity for orientation and for the direction of movement are absent at P17 and they increse progressively to reach adult values at around P30.

Receptive fields (RF) in adult rats are small and well defined, but this is not the case of younger animals. At P17, RF are very large, they extend through almost all the binocular hemifield and they are difficult to define since cell responses are weak and tend to habituate. At P19-21 RF size is around 34 degrees (deg), while it reaches the values of 10 deg or less in the adult.

The visual acuity is about 0,5 cycles/degrees (c/deg) at P17 whereas it reaches the value of 1,0-1,2 c/deg in the adult. It has to be noticed that development of visual acuity is correlated with the decrease of the mean RF size (Fagiolini et al., 1994).

Plasticity in the visual cortex

The visual cortex has been a model for plasticity in the CNS since the pioneering work of Wiesel and Hubel (1963). Still now, it is considered one of the best example of the nature-nurture interaction during brain development and, due to the relative simplicity of triggering sensory alteration in the visual system, it can be used as a paradigm of experience-dependent modifications of neuronal connections.

Visual system plasticity and amblyopia

One of the best described pathologies of the visual system development is amblyopia. Also known as lazy eye, it has traditionally been defined as a decrease of visual acuity caused by pattern vision deprivation or abnormal binocular interaction for which no causes can be detected by the physical examination of the eye.

Amblyopia is estimated to afflict 1-4% of children, with recent large population studies falling in the range of 1.6-3.6%, and with evidence that the rate is even higher in medically underserved populations (for a review see Simons, 2005).

The problem is caused by either no transmission or poor transmission of the visual image to the brain for a sustained period of dysfunction or during early childhood. Amblyopia normally only affects one eye, but it is possible to be amblyopic in both eyes if both are similarly deprived of a good, clear visual image.

This pathology can be distinguished in three main types depending on its etiology. It can be caused by deprivation of vision (vision-obstructing disorders such as congenital cataracts), by strabismus (misaligned eyes), or by anisometropia (different degrees of myopia or hyperopia in each eye).

As already said, the functional abnormalities have long been attributed to abnormal visual experience in early life, and for this reason Hubel and Wiesel started

investiganting the effects of ocular occlusion and strabismus on the LGN and visual cortex of cats very soon after they had established the normal properties of single neurons recorded in these places. Their first experiment was to occlude, by suturing the eyelids, one or both eyes of kittens as soon as they opened (monocular deprivation, MD). Several weeks or months later they reopened the occluded eye and recorded from single neurons in the LGN or visual cortex.

Due to the decussation of retinal inputs, cells recorded from a normal visual cortex can be classified according to their relative response to the stimulation of the two eyes. Thus, Hubel and Wiesel indicated with the class 1 the cells that can be excited only by the contralateral eye, with class 7 the cells guided exclusively by the ipsilateral eye and with class 4 the cells equally driven by either eye. The other classes correspond to cells judged to have intermediate degrees of dominance of each eye.

With this paradigm Hubel and Wiesel demonstrated that closing one eye at the time of eye opening dramatically decreases the number of neurons of the visual cortex that respond to that eye and cortical cells become strongly dominated by the non-occluded eye.

Thus, apparently the disuse of an eye weakens the connections it makes with the LGN and visual cortex. Since usage of the pathway appears to be necessary to preserve normal functions, it may be thought that suture of both eyelids, or total visual deprivation by rearing in the dark, would produce even greater changes than those described. Nevertheless, these two treatments do not cause any change in term of ocular dominance distribution. This evidence has been classically interpreted calling into question a form of competition of the two eyes. In other words, the pathway from one eye to a cortical neuron is not disconnetted or impaired, unless there is another pathway from the other eye competing for control of the same cortical neuron.

This competition leads not only to physiological but also to anatomical rearrangements. For example, in the lateral geniculate nucleus of rodents it has been observed a reduction in the number of projections to the visual cortex contralateral to the deprived eye accompanied by an hypotrophy of the neuronal cellular bodies (Domenici et al. 1993; Antonini et al. 1999).

MD effectiveness in modifying the ocular dominance (OD) of visual cortical

neurons and in producing detectable morphological changes declines with age (Hubel and Wiesel 1970; Fagiolini et al. 1994; Prusky et al. 2000; Mataga et al. 2002; Prusky and Douglas 2003; Liao et al. 2004; Mataga et al. 2004). Even in the mouse, in which a greater potential for experience-dependent plasticity in the adult visual cortex has been recently demonstrated (Sawtell et al. 2003), plasticity in response to short term (3 days) MD is restricted to the critical period (for a review see Morishita and Hensch, 2008). Moreover, It has been demonstrated that if the visual defect is removed early in development (for example through reverse suture, i.e. opening of the formerly closed eye and deprivation of the open eye), the ensuing recovery is very good; on the contrary, recovery is very limited if defect removal is delayed to adulthood.

Despite decades of research, the cellular and molecular factors contributing to the decline in plasticity after the critical period are still poorly understood (see Berardi et al. 2003). In the last few years, several candidates have emerged as critical in regulating developmental plasticity: the maturation of myelin (Mc Gee et al. 2005), the condensation of extracellular matrix molecules into perineuronal nets (Hockfield et al 1990; Pizzorusso et al. 2002) and the maturation of intracortical inhibition (Hensch et al. 1998; Huang et al. 1999; Hensch 2005). In particular, several papers converged in indicating the last two factors as key components not only for the regulation of critical period but also for the restoration of plasticity in the adult visual system.

Extracellular matrix and plasticity

A substantial amount of brain volume consists of extracellular space interposed between brain cells. This space is filled with a matrix of molecules interconnected and linked with membrane bound molecules. These interactions are essential for the mechanical properties of brain tissue and are also able to activate intracellular signaling pathways. A number of studies, reviewed by Dityatev and Schachner (2003), have involved elements of this network, such as integrins, cadherins, NCAM, tenascins, and heparinsulfate proteoglycans, in synaptic plasticity (LTP and LTD) and in learning and memory processes. Furthermore, several studies have shown an important role for key components of the brain extracellular matrix (ECM), the chondroitin-sulfate proteoglycans (CSPGs), in OD plasticity of the visual cortex. CSPGs are major con-

stituents of the ECM of the CNS and comprise a core protein and chondroitin-sulfate glycosaminoglycan chains. CSPGs are inhibitory for axonal sprouting, and after injury they are upregulated in the CNS, with the effect of blocking axon regeneration (Bradbury et al., 2002; Silver and Miller, 2004). During development, CSPGs condense at high concentration in lattice-like structures, denominated perineuronal nets (PNNs), which completely envelop visual cortical neurons.

PNNs are fenestrated at sites of synaptic contact, where they assume a perisynaptic localization (Dityatev and Schachner, 2003). The process of condensation of CSPGs into PNNs begins during late development and is completed after the end of the critical period. Dark rearing, which prolongs critical period closure, also prolongs CSPGs condensation (Hockfield et al., 1990). Degradation of CSPGs from the adult visual cortex with the enzyme chondroitinase ABC reactivates ocular dominance plasticity in monocularly deprived adult rats, suggesting that the CSPG-enriched adult ECM exerts a powerful repressive control on OD plasticity (Pizzorusso et al., 2002). This could be partially due to a non-permissive action on the remodeling of visual cortex pyrmidal neurons, as suggested by the well documented effect of the ECM on spine motility (Majewska and Sur 2003; Mataga et al. 2004; Oray et al. 2004; Pizzorusso et al. 2006). However, as already said, toward the end of the critical period CSPGs condense at an extremely high concentration in PNNs surrounding parvalbumin-positive inhibitory interneurons (Hartig et al. 1992, 1999). Therefore, at least some of the effects of chondroitinase ABC could be mediated by modifications of intracortical inhibitory circuits occurring after PNNs degradation. This is a very interesting point, since it establishes a link with a second crucial regulating factor of plasticity, the inhibitory system.

Inhibition and plasticity

GABA (γ-aminobutirric acid) is the most abundant inhibitory neurotransmitter in the brain. It is synthesized by two distinct isoform of glutamic acid decarboxylase, GAD65 and GAD67 (Soghomonian and Martin 1998). The latter is the primary synthetic enzyme, it is localized to cell bodies and its deletion is lethal causing death at birth. The other isoform, GAD65, is concentrated in axon terminals and bound to

synaptic vescicles. GAD65 knockout mice are viable even if both overall GABA levels, and the amounts released by stimulation, are markedly reduced. Although the cortex is not epileptic (as occurs when inhibition is totally eliminated) neurons in these mice show prolonged responses to visual stimuli, suggesting that the inhibitory circuits are less effective. Interestingly, Hensch et al. (1998) found that these GAD65 knockout mice showed no shift in electrophysiologically measured ocular dominance preference when one eye was closed during the critical period. Remarkably, local infusion of diazepam, a use-dependent GABA agonist, restored the ability of neurons to undergo an ocular dominance shift in response to eye closure, starting a normal critical period; this rescue of plasticity was possible at any age, which indicates that the beginning of the critical period is dependent on the proper level of inhibitory transmission. Conversely, using the same approach, Fagiolini and Hensch (2000) provided strong evidence that increased intracortical inhibition is a major factor responsible for triggering the initial onset of the critical period. Indeed, premature enhancement of inhibition with diazepam just after the eye opening triggers the onset of a precocious critical period in normal animals. Importantly, once diazepam induced plasticity has occurred in a given animal, no further ocular dominance plasticity can occur later in life, suggesting again that diazepam initiate a normal critical period that, once overtaken, prevents subsequent plasticity.

Another evidence comes from the BDNF transgenic mice generated by Huang and colleagues. In these mice, BDNF overexpression accelerates the development of interneurons, thus increasing inhibition levels earlier than in wild-type animals (Huang et al., 1999). Interestingly , it has been shown by single cell extracellular recordings that, in these mice, the critical period for ocular dominance plasticity starts earlier than in wild-type (Hanover et al., 1999).

A close relationship between neural activity, BDNF release and GABA function also explains the classic effect of dark-rearing. Raising animals in darkness from birth reduces BDNF levels (Castren et al., 1992) and development of cortical inhibition (Benevento et al., 1995) and delays the peak of plasticity into adulthood. Diazepam infusion (Iwai et al., 2003), BDNF overexpression (Gianfranceschi et al., 2003) or secretion by environmental enrichment (Bartoletti et al., 2004) abolishes the delay of

critical period triggered by darkness. Together, all these striking results seem to suggest that during development, the inhibitory tone surpasses two functional thresholds in the visual cortex: the first one that enables plasticity probably being necessary to detect activity differences between the two eyes, the other that subsequently closes the critical period by further reducing activity levels (Feldman, 2000).

Interestingly, a certain amount of inhibition is not only needed to close critical period but it is still effective during adulthood in limiting plasticity. Indeed, in a recent study by our group it has been demonstrated that the pharmacological reduction of intracortical inhibition reactivates ocular dominance plasticity in response to monocular deprivation in adult rats (Harauzov et al., in press). This could have implications also for brain repair, making intracortical inhibition a target for behavioural or pharmacological interventions in presence of brain lesions. A change in inhibitory tone has indeed been found in perilesional regions in the visual cortex and also in patients with stroke in the motor cortex (Liepert, 2006). Moreover the hippocampal learning and memory deficits of an animal model of Down's syndrome (the Ts65Dn mice) appear to be due to enhanced GABAergic inhibition (Kleschevnikov et al. 2004). A recent ground-breaking study (Fernandez et al. 2007) showed that although acute treatment (1 day) with GABAA receptor antagonist (picrotoxin) had no effect, a 2 week treatment with several GABAA receptor antagonist (picrotoxin pentylenetetrazole or bilobabide) rescued cognitive deficits in adult Ts65Dn mice. Surprisingly, it was shown that the behavioural and phisiological recovery was persistent, lasting for at least two months after the end of treatment.

The shift toward higher inhibition in this model of neurodevelopmental disorder raises the possibility for premature closure of critical period in these mutant mice, a mechanism that could contribute to their deficits. Accordingly, strategies that decrease inhibition may reactivate developmental levels of plasticity in the adult brain (Spolidoro et al. 2009).

The work by Harauzov and collegues pointed out another important result. The reactivation of ocular dominance plasticity in the adult visual cortex caused by the reduction of intracortical inhibition is accompanied by rearrangements in the extracellular matrix (ECM) with a reduction in perineuronal nets. Together with the

evidence that in the CA1 region of the hippocampus the disruption of PNNs is associated with a reduction in perisomatic inhibition (Saghatelyan et al., 2001), this result strongly suggests that a crosstalk between the two systems probably exist and that both must be taken into account looking for possible strategies to recover plasticity in the adulthood.

Although the above mentioned work is the first direct evidence that the inhibitory system has a fundamental role in reducing plasticity in the adult, several recent papers have shown that the restoration of plasticity to adulthood is often accompanied by a change in the inhibitory/excitatory balance in the brain. Exposure of adult rats to complete darkness enhances experience-dependent visual cortical plasticity, leading to a reduced expression of GABAA receptors relative to AMPA receptors, thus altering the balance between inhibition and excitation in the visual cortex (He et al., 2006).

In the following section we will discuss very recent results in which two different strategies, namely environmental enrichment and chronic treatment with fluoxetine, have been shown to restore plasticity in adulthood by a reduction of the inhibitory tone. If this reduction was counteracted, restoration of plasticity was prevented, demonstrating that a reduced inhibition is necessary for the plastic outcome at the functional level.

Environmental Enrichment and fluoxetine treatment: two different methods to enhance plasticity and to reduce inhibition

Environmental enrichment (EE) is a widely used paradigm to investigate the influence of sensory experience on brain and behaviour (for a review see Rosenzweig and Bennett 1996; van Praag et al 2000; Diamond 2001). Enriched animals are reared in large groups in wide cages where a variety of toys, tunnels, nesting materials and stairs are present and changed frequently. In addition, animals are typically given the opportunity for voluntary physical activity on running wheels.

This definition of EE is based on the comparison of the enriched condition with alternative conditions frequently used in the laboratory, the standard condition, in which animals are reared in little groups of individuals in small cages where no particular objects other than nesting material, food and water are present, and the so-called

impoverished condition, in which even normal social interactions are prevented because animals are reared in isolation. The naturalistic environment recreated in the enriched condition gives animals the opportunity for enhanced social interactions, a continuous multi-sensorial stimulation provided by the novelty of new objects attracting the explorative curiosity of most laboratory animals and allows the organism to benefit from a balanced schedule of feeding and physical activity.

Rearing animals in an enriched environment has profound effects on the development of the nervous system. Indeed, it has been demonstrated that EE from birth leads to a profound acceleration of important properties of visual system development at behavioural, electrophysiological and molecular level (Cancedda et al., 2004; Sale et al., 2004; Ciucci et al. 2007) and accelerates the postnatal development of the retina (Landi et al., 2007; Sale et al. 2007b). Furthermore, it affects BDNF expression in the visual cortex at very precocious ages (Cancedda et al., 2004).

Environmental enrichment has strong effects also on the adult organism, leading to anatomical changes (observed, for instance, in the cortex, hippocampus and cerebellum) in dendritic arborization, spine density and number of synapses per neuron (Rosenzweig, 1966; Greenough and Volkmar, 1973; Renner and Rosenzweig, 1987 and Rampon et al., 2000). These morphological changes are associated with improved learning and memory and enhanced neural plasticity (reviewed in van Praag et al., 2000) and reorganization of cortical somatosensory maps (Polley et al., 2004). Moreover, EE has been demonstrated to be beneficial in reducing cognitive deficits and the progression of the disease in several models of neurodegenerative pathologies related to human diseases, such as Huntington's and Alzheimer's, and in preventing neurodegeneration caused by ischemic or traumatic insults (Lazarov et al., 2005; Nithianantharajah and Hannan, 2006; Berardi et al., 2007).

Only recently, environmental enrichment was explored as a strategy to enhance plasticity in the adult. It has been shown that EE promotes a complete recovery of visual acuity and ocular dominance in adult amblyopic animals (Sale et al., 2007a). Recovery of plasticity was associated with a marked reduction of GABAergic inhibition in the visual cortex, as assessed by in vivo brain microdyalisis. Moreover, a decreased cortical inhibition has been demonstrated also at the synaptic level, using the in vitro

paradigm of long-term potentiation of layer II-III field potentials induced by theta-burst stimulation of the white matter (WM-LTP). This form of LTP is normally not present in the adult as a result of the maturation of inhibitory circuits (Kirkwood and Bear 1994; Huang et al. 1999), but it can be restored if GABA.mediated inhibition is reduced (Artola and Singer 1987; Kirkwood and Bear 1994). Notably WM-LTP was fully restored in the visual cortex of EE adult rats (Sale et al. 2007a). The authors also demonstrated that the reduction of inhibition is a crucial molecular mechanism underlying the enhancement of plasticity induced by EE, since restoration of plasticity was completely prevented by benzodiazepine cortical infusion during EE period. The reduction of cortical inhibition in EE rats was also paralleled by an increased expression of the neurotrophin BDNF and a lower density of PNNs in the visual cortex contralateral to the recovering (previously amblyopic) eye.

It is well known that EE has a profound effect on diffuse projections' system in the brain, increasing acetylcholinesterase activity, noradrenalin and serotonin (Rosenzweig et al. 1962, 1967; Escorihuela et al. 1995; Rasmuson et al. 1998; Naka et al. 2002). Since these neuromodulators have been reported to influence plasticity in the adult brain, it would be interesting to test whether it is possible to reproduce the EE effects on adult visual cortex plasticity by an artificial modulation of their levels.

This possibility was addressed recently by Maya Vetencourt and collegues (2008) The authors found very similar results to those reported for EE using chronic treatment with fluoxetine, a selective serotonin reuptake inhibitor (SSRI) widely prescribed in the treatment of depression.

Clinically used antidepressant drugs (ADs) increase extracellular serotonin and/or norepinephrine levels, but the relationship between acute increases in these neurotrasmitters and the clinical antidepressant effect , which develops with a time delay of several weeks, lies unclear (Nestler, 1998; Castren, 2005). Chronic antidepressant administration promotes neurogenesis and synaptogenesis in the adult hippocampus (Malberg et al., 2000; Hajszan et al., 2005) as well as increased expression of the neurotrophin BDNF and its primary receptor TrkB (Nibuya et al., 1995; Castren, 2004). These cellular and molecular events seem to be necessary to mediate the therapeutic effects of antidepressants. The behavioural response to ADs is blocked if the induced

neurogenesis is disrupted (Santarelli et al., 2003), whereas direct infusion of BDNF into the hippocampus or the overexpression of its receptor in transgenic mice induces an antidepressant effect (Shirayama et al., 2002; Koponen et al., 2005).

Recent experimental evidences also suggest that a disturbance of brain plasticity is involved in animal models of depression and that chronic antidepressant treatment may counteract these alterations. Indeed chronic mild stress facilitates LTD in the hippocampus of adult rats and has no effect on LTP, while treatment with ADs prevents the induction of LTD and increases the extent of LTP (Holderbach et al., 2007).

Taken together, these data suggest an influence of antidepressant in neuronal plasticity. However, as mentioned before, in a very recent work this influence was investigated directly. In fact, in the work by Maya-Vetencourt it has been demonstrated that chronic treatment with fluoxetine, restores platicity to the adult visual system of the rat. This was assessed using two classical models of plasticity: 1) the ocular dominance shift of visual cortical neurons after MD and 2) the recovery of the visual function in the amblyopic adults (Maya Vetencourt et al., 2008).

Fluoxetine-treated rats displayed a coplete shift in OD in response to MD assessed by VEPs. Inportantly this shift was not due to an enhanced response to the open eye (as in the case of OD palsticity in adult mice; Sawtell et al. 2003; Frenkel and Bear 2004), but to a reduction of VEPs amplitude elicited by stimulation of the deprived eye, as tipically observed in juvenile cortex. Fluoxetine exerted also a powerful effect as a therapeutic agent for amblyopia, leading to the complete recovery of normal visual functions in long-term monocularly deprived animals (Maya-Vetencourt et al. 2008). As found for EE, the effects induced by fluoxetine in adult visual cortex plasticity were associated with a marked reduction of GABAergic inhibition. Once more, cortical diazepam administration totally prevented the OD shift induced by MD in adult rats chronically treated with fluoxetine.

The fact that both EE and fluoxetine administration lead to reduced GABAergic neurotransmission highlights the importance of the intracortical inhibitory tone as a key element regulating visual cortex plasticity in the adult brain. Both treatments, in addition, have been shown to increase BDNF expression, a critical factor for experience-dependent plasticity. Thus, these results may be explained through a

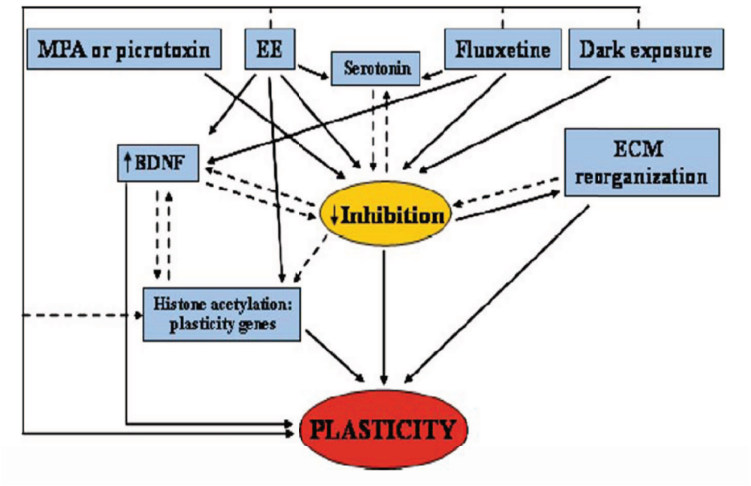

Figure 1: Schematic diagram showing key molecular events underlying restoration of plasticity in the adult visual system. Maturation of the inhibitory circuitry is thought to be one of the most important limiting factors that restrain visual cortex plasticity to the critical period. The restoration of visual cortex plasticity in adult animals is accompanied by a reduction in intracortical inhibition in different paradigms as MPA treatment, EE, chronic administration of SSRIs and dark exposure. We propose a model in which a decreased level of inhibition is the central hub triggering plasticity in the adult visual cortex. The reduction in the inhibitory tone may be accompanied by a reorganization of the ECM and an enhancement of BDNF expression. ECM remodeling could be the starting point for structural modifications at the level of synaptic connectivity, while BDNF could upregulate other genes that promote plasticity. Furthermore, an influence on the epigenetic control of gene transcription has been suggested at least for EE. Histone acetylation could be the final gate to reopen plasticity in the adult visual cortex. Continuous lines represent well-documented interactions between boxes; dashed lines indicate likely interactions in the context of visual cortical plasticity deserving further experimental characterization

model in which enhanced sensory-motor activity under environmental enrichment or enhanced serotoninergic transmission elicited by SSRI treatment, decrease cortical levels of inhibition and, in parallel or in series, increase BDNF expression, which could in turn upregulate other genes that promote plasticity (fig.1).

Interestingly, it has been recently demonstrated that EE has an influence on chromatine remodeling. Histone posttranslational modifications regulate chromatin susceptibility to transcription with high level of histone acetylation on a specific DNA segment being generally correlated with increased transcription rates (Mellor 2006; Workman 2006). EE enhances histone acetylation in the hippocampus and, to a lesser extent, in the cortex of P25 mice (Fischer et al. 2007) rescuing the ability to form new

memories and re-establishing access to remote memories even in the presence of brain degeneration. A similar relationship between histone acetylation and EE effects couls also occur in the adult visual system. Visual experience activates histone acetylation in the visual cortex during the critical period, but this capacity is downregulated in adult animals (Putignano et al. 2007). Trichostatin treatment, which promotes histone acetylation, also enhances plasticity in the adult visual cortex (Putignano et al. 2007). Thus, it is possible that the cellular and molecular mechanism proposed by Sale et al. (2007a) to mediate the effects of EE on the adult visual system could ultimately regulate the pattern of histone acetylation, modulating the expression of genes crucial for plasticity.

Food restriction and brain health

The influence that dietary factors have on the function of the nervous system, and on its susceptibility to disease, is an active and important area of biomedical research. Studies have identified several specific dietary components that are critical for the proper development of the nervous system. Folate deficiency during pregnancy can result in neural tube defects in babies (Refsum 2001), choline deficiency during brain development may result in learning and memory problems (Zeisel 1997), and certain fatty acids are critical for optimum brain function in the adult (Chalon et al. 2001). Recent findings suggest that folate deficiency (and a consequent increase in the levels of homocysteine) may increase the risk of Alzheimers disease (AD), Parkinsons disease (PD), stroke and psychiatric disorders (Duan et al. 2002; Kruman et al. 2002; Seshadri et al. 2002). Folate plays a critical role in one-carbon metabolism by facilitating the remethylation of methionine from homocysteine (Fenech 2001). By increasing homocysteine levels and impairing DNA synthesis, methylation and repair, folate deficiency can damage cells including neurons (Kruman et al. 2000, 2002).

Other examples of dietary supplements that may improve brain function and/or protect against age-related disease include antioxidants such as vitamin E (Halliwell 2001), Ginko biloba extract (Youdim and Joseph 2001) and creatine (Sullivan et al. 2000).

While vitamins, minerals and antioxidants may improve the healthspan of the brain, a more fundamental aspect of diet is emerging as a major factor in brain health. This factor, which is the focus of the present chapter, is caloric intake.

Some notes on metabolism

Metabolism is the set of chemical reactions that occur in living organisms in order to maintain life. These processes allow each individual to grow and reproduce, maintain its structure, and respond to its environment. Metabolism is usually divided in two categories. Catabolism breaks down organic matter, while anabolism uses energy to construct components of cells such as proteins and nucleic acids.

The chemical reactions of metabolism are organized in metabolic pathways, in which one chemical is transformed into another by a sequence of enzymes. Enzymes are crucial to metabolism because they allow organisms to drive desirable but thermodynamically unfavorable reactions by coupling them to favorable ones, and because they act as catalysts to make these reactions proceed quickly and efficiently. Enzymes also allow the regulation of metabolic pathways in response to changes in the cell's environment or signals from other cells.

Most of the structures that make up animals, plants and microbes are made from three basic classes of molecule: amino acids, carbohydrates and lipids (often called fats). As these molecules are vital for life, metabolism focuses on making these molecules, in the construction of cells and tissues, or breaking them down and using them as a source of energy. Many important biochemicals can be joined together to make polymers such as DNA and proteins. These macromolecules are essential parts of all living organisms.

The carbohydrates, fats and proteins in food are metabolized to glucose which is then utilized as the major source for ATP production in cells. Unlike other cells which can use different fuels for a period of time (for example fatty acids), the only source of energy for brain cells is glucose. Moreover, because neurons cannot store this sugar, they depend on the blood stream to deliver a constant supply of this precious fuel. For this reason, blood glucose levels normally needs to remain within a proper range.

The homeostatic mechanism which keeps the blood value of glucose in a remarkably narrow range is composed of several interacting systems, of which hormone regulation is the most important. The two main hormones implicated in this phenomenon are insulin and glucagon.

These two hormones are secreted by islet cells within the pancreas and work antagonistically. Insulin is normally secreted by the β cells and the stimulus for its secretion is a high level of blood glucose. Similarly, as blood glucose falls, the amount of insulin secreted by the pancreatic islets goes down. This hormone has an effect on a number of cells, including muscle, red blood cells, and fat cells. In response to insulin, these cells absorb glucose from bloodstream, having the net effect of lowering the high blood glucose levels into the normal range.

Conversely, glucagon is secreted by the α cells of the pancreatic islets when blood glucose diminishes (for example, between meals and during exercise). It has an effect on many cells type but it influences mostly the liver function. Indeed, its principal role is to promote the release of glucose from liver storage, with the net effect of increasing blood glucose.

In addition to these mechanisms, during long periods of fasting the organism activate the so called stress response which involves the stimulation of the hypothalamic-pituitary-adrenal axis (HPA axis). This is a complex set of direct influences and feedback interactions among the hypothalamus, the pituitary gland, and the adrenal glands. Briefly, in response to almost any type of stress physical or psychological, cells in hypothalamus produce the corticotrophin-releasing factor (CRF) which binds to specific receptors on pituitary cells stimulating the production of adrenocorticotropic hormone (ACTH). ACTH is then transported to the adrenal gland and promotes the production of cortisol (corticosterone in rodents). The loop is completed by the negative feedback of cortisol on the hypothalamus and pituitary.

The simultaneous release of cortisol has a number of effects, including elevation of blood glucose for increased metabolic demand. Indeed, one of the actions of cortisol is to counteract insulin producing gluconeogenesis and promoting breakdown of lipids (lipolysis), proteins, and mobilization of extrahepatic amino acids and ketone bodies.

Beneficial effects of food restriction

Both the number of calories consumed over time and the time interval between feedings affect the physiology of brain cells in quite profound ways. The dietary restriction (DR) protocols used in the animal studies, indeed, involve a reduction in overall

calorie intake, and/or an increase in the intermeal interval, with maintenance of the composition of the diet in terms of vitamins, minerals, protein, etc. In other words, in a first kind of procedure the animals receive daily a quantity of food that is 30-60% of the amount consumed by the controls fed ad libitum, this protocol is better known with the term of caloric restriction (CR); the second paradigm, called intermittent fasting (IF), involves a fasting regimen on alternate days, i.e. the animals have not access to food for a full day, every other day, while they are allowed to eat ad libitum on the intervening days. The expression of food restriction (FR) refers to both methods indistinctly.

Lifespan and susceptibility to desease. The first widely recognized scientific study of restricted diets was published by McCay et al. (1935). They showed that feeding rats with a diet containing indigestible cellulose dramatically prolonged both mean and maximum life-span in these animals. Since then, many studies have confirmed this result and extended it to mice (Weindruch and Walford, 1988; Sprott, 1997) and other species including fruitflies (Chapman and Partridge, 1996) nematodes (Houthoofd et al., 2002), water fleas, spiders and fish (Weindruch and Walford, 1988).

FR reduces the incidence of age-related cancers, cardiovascular desease an deficits in immune function in rodents (Weindruch and Sohal, 1997). Conversely, overeating is a risk factor for cardiovascular desease, many type of cancers, type-2 diabetes and stroke (Levi, 1999; Brochu et al., 2000). Less documented is the evidence suggesting that FR reduces desease risk and extends lifespan in indivuduals that are not overweight (Roth et al., 2002; Walford et al., 2002).

The FR regimens have also been shown to have beneficial effects on the brain. For example, FR retards age-related increases in the levels of glial fibrilary acidic protein and oxidative damage to proteins and DNA (Dubey et al., 1996; Major et al., 1997). Analysis of the levels of mRNAs encoding thousands of proteins in the brains of young and old rats, which had been fed either ad libitum or FR diets, revealed numerous age-related changes in gene expression that were attenuated by FR (Lee et al., 2000a). The genes whose expression was affected by ageing and counteracted by FR included those involved in oxidative stress responses, innate immunity and energy

metabolism.

Synaptic plasticity and neuorgenesis. Because deficits in learning and memory, motor control and other behaviours occur during ageing, some of the earliest studies of the impact of FR on the nervous system involved testing the function of the nervous systems of old rodents that had been mantained on ad libitum or calorie-restricted diets during their adult lives.

Mice mantained on a diet with a 40% reduction in calories beginning at the time of weaning did not exhibit the deficits in motor coordination and spatial learning seen in control mice fed ad libitum (Ingram et al., 1987). FR beginning at 3 months of age prevented age-related deficits in a radial maze learning task in mice (Idrobo et al., 1987). Similarly, life-long CR prevented age-related deficits in the performance of rats in radial arm maze and Morris water maze learning and memory tasks (Stewart et al., 1989). FR retarded age associated deficit in sensorimotor coordination and avoidance learning in mice (Dubey et al., 1996).

Long-term potentiation (LTP) of synaptic transmission is believed to be a cellular correlate of learning and memory. Aged rats exhibit a deficit in LTP in the hippocampus, and this deficit is largely abolished in age matched rats that are fed a reduced calorie diet during their adult life (Hori et a., 1992; Eckles-Smith et al., 2000). Moreover, beneficial effects of FR were evident in aged (22-month-old) mice in which caloric restriction was initiated in mid-life (14 months of age); strenght and coordination were preserved such as age related changes in spontaneous alternation behaviour (Means et al., 1993).

A few studies have examined synapses from animals that had been maintained of FR. In one study, neocortical synaptosomes were isolated from rats under IF and from controls. The synaptosomes from FR rats exhibited improved glucose tranport and mitochondrial function following exposure to oxidative and metabolic insults (Guo et al., 2000).

Effects of FR on neurotransmitters have also been documented. For example, FR prevented age-related alterations in the levels of serotonin and dopamine in the cerebral cortex of rats (Yeung and Friedman, 1991), and enhanced evoked dopamine

accumulation in the striatum of aged rats (Diao et al., 1997). Preservation of neurotransmitter signaling is likely to be crucial for the ability of FR to maintain the function of the nervous system during ageing.

The adult brain contains populations of cells that are capable of dividing and then differentiating into neurons (neurogenesis) or glial cells (gliogenesis) (Gage, 2000). In mammals, including humans, neural stem cells are most abundant in the subventricular zone and the dentate gyrus of the hipocampus. Stem cells in the adult brain may provide a cellular reserve to replace neurons and glia that die as the result of various injuries and deseases; evidence suggesting that neurogenesis can be stimulated by ischemic and excitotoxic brain injuries is consistent with the cellular reserve hypothesis (Parent et a., 1997; Liu et al., 1998).

Interestingly, more subtle physiological signals can regulate neurogenesis, suggesting that neural stem cells may be continuously responding to functional demands. For example, raising rats or mice in an enriched environment or increasing their level of physical exercise can enhance neurogenesis (Kemperman et al., 1997; Nilsson et al., 1999; van Praag et al., 1999). In addition, neurogenesis and synaptic connections are affected by changes in the levels of the sex steroids testosterone and estrogen (Alvarez-Buylla and Kirn, 1997; McEwen, 2001).

It has been reported that FR can increase neurogenesis in the brains of adult rats and mice (Lee et al., 2000b; 2002 a,b) promoting the survival of newly generated neural cells.

Neuroprotection. The number of people with age related neurodegenertive conditions such as Alzheimer's desease (AD), Parkinsons desease (PD), stroke and hearing and vision loss is increasing rapidly as life expectancy continues to raise.

Different but overlapping populations of neurons degenerate in AD, PD and stroke. Neurons in brain regions involved in learning and memory processes, such as the hipocampus and the cerebral cortex, are affected in AD (Ray et al., 1998). In PD dopaminergic neurons in the substantia nigra degenerate resulting in motor dysfunction (Jenner and Olanow, 1998). A stroke occurs when a cerebral blood vessel becomes occluded or rupted resulting in the degeneration of neurons in the brain tissue supplied

by that vessel (Schulz and Dichgans, 1999).

Overeating is a well established risk factor for stroke, and some epidemiological data suggest that individuals with high caloric intake may also be at increased risk for AD and PD (Bronner et al., 1995; Logroscino et al., 1996; Mayeux et al., 1999).

A series of studies have employed animal models of neurodegenerative disorders to directly determine the effects of FR on neuronal vulnerability and functional outcome; the models are based on genetic and environmental factors that may initiate or promote the neurodegenerative process in the corresponding human disorder. AD models include transgenic mice expressing mutant forms of human amyloid precursor protein and/or presenilin-1 that cause early onset inherited AD (Games et al., 1995; Duff et al., 1996; Hsiao et al., 1996; Guo et al., 1999) and infusion of amyloid ß-peptide and excitotoxins into the brains of rats and mice (Geula et al., 1998; Bruce-Keller et al., 1999). PD models include administration of the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxy-dopamine or rotenone to rodents or monkeys resulting in the selective degeneration of substantia nigra dopaminergic neurons and associated motor dysfunction (Duan et al., 1999), and transgenic mice expressing mutant human a-synuclein, which exhibit degeneration of dopaminergic neurons and a behavioral phenotype that mimicks several features of PD (Masliah et al., 2000). A stroke can be induced in rodents by transient or permanent occlusion of the middle cerebral artery (Dirnagl et al., 1999; Yu and Mattson 1999). Many of the neuronal deaths that occur in these animal models are believed to involve a form of programmed cell death called apoptosis (Mattson, 2000).

Rats maintained on FR for 2-4 months exhibit increased resistance of hippocampal neurons to excitotoxic degeneration in a model relevant to the pathogenesis of epilepsy and AD; this neuroprotection resulted in a preservation of learning and memory ability that is normally compromised in this model (Bruce-Keller et al., 1999). In addition to its neuroprotective actions, FR may be beneficial for epilepsy patients by reducing seizure incidence and severity (Mahoney et al., 1983; Greene et l., 2001; Yudkoff et al., 2001).

In other studies, presenilin-1 mutant knockin mice and amyloid precursor protein (APP) mutant transgenic mice were maintained on FR or ad libitum control diets.

It has been demonstrated that presenilin-1 mutations increase the vulnerability of hippocampal and cortical neurons to excitotoxicity and apoptosis by a mechanism involving enhanced calcium release from the endoplasmic reticulum (Guo et al., 1999). This effect was couteracted by FR and levels of oxidative stress was decreased in presenilin-1 mutant mice under dietary regimen (Zhu et al., 1999).

Also the vulnerability of nigro-striatal dopaminergic neurons to MPTP toxicity was decreased in mice maintained on FR with increase in survival of cells and attenuation of motor function deficits (Duan and Mattson, 1999). The striatal pathology in Huntington's disease (HD) patients can be partially reproduced in rats by administration of the succinate dehydrogenase inhibitor (mithocondrial toxin), 3-nitropropionic acid. When rats were subjected to FR for several months prior to administration of the toxin, more striatal neurons survived exposure to the drugand their motor function was improved dramatically (Bruce-Keller et al., 1999).

The ability of FR to improve outcome after a stroke was demonstrated in a rat model in which the middle cerebral artery is transiently occluded resulting in damage to the cerebral cortex and striatum, and associated motor dysfunction (Yu and Mattson, 1999). when rats were maintained on a periodic fasting regimen for several onths they exhibited reduced brain damage and improved behavioral outcome following transient focal ischemia.

The evidence that FR can reduce the risk of neurodegenerative disorders is supported by epidemiological studies on humans. There is an inverse correlation between the incidence of AD throughout different populations of the world and their average daily food intake (Grant, 1999). Thus, people in China and Japan have relatively low calorie intakes (1600/2000 calories/day) as compared with people in the United States and Western Europe (2500/3000 calories/day), and the incidence of AD in China and Japan is approximately half that seen in US and Western Europe. Although there are otential confounders in such analyses (for example, per capita food consumption is a very poor measure of energy intake, and disease diagnosis may differ among the countries), they are consistent with a protective effect of low calories diets against age related neurodegenerative disorders.

Molecular mechanisms of neuroprotection induced by FR

Data from the animal studies described show that neurons in the brains of rats and mice maintained on CR or IF regimens exhibit increased resistance to oxidative, metabolic and excitotoxic insults. The critical question to ask with respect to these studies is, what are the underlying molecular mechanisms that account for the protection against this myriad of potent cellular insults? Investigators have addressed this important question by measuring numerous proteins and lipids that are known to play a role in protecting neurons against many different insults.

Stress responses. The acquisition of available food forms one of the most profound behavior sets. Thus, removal of adequate food sources acts as a great driving force for behavioral changes and causes a certain degree of psychological and physiological stress in the organism.

To analyse this aspect, several different stress proteins, including heat-shock and glucose-regulated proteins, have been measured in the brains from rats maintained on either *ad libitum* or FR diets. These molecular chaperones interact with many different proteins in cells and function to ensure either their proper folding or their degradation when they are damaged (Frydman, 2001; Gething, 1999). They may also interact and modify the function of apoptotic proteins including caspases (Beere et al., 2000; Ravagnan et al., 2001). Levels of some of these chaperones may be increased during the aging process as a protective response (Lee et al., 1999, 2000a,b). Cell culture and in vivo studies have shown that heat-shock protein-70 (HSP-70) and glucose-regulated protein 78 (GRP-78) can protect neurons against injury and death in experimental models of neurodegenerative disorders (Lowenstein et al., 1991; Yu and Mattson, 1999). Levels of HSP-70 and GRP-78 were found to be increased in the cortical, hippocampal and striatal neurons of the FR rats compared to the age-matched ad libitum fed animals (Lee et al., 1999). Previous studies have provided evidence that HSP-70 and GRP-78 can protect neurons against excitotoxic and oxidative injury (Warrick et al., 1999; Yu et al., 1999), which suggests that they contribute to the neuroprotective effect of FR. These data may demonstrate that FR can induce a mild stress response in neurons, presumably due to a reduced energy, primarily glucose, availability. Indeed, in addition

to these subcellular stress responses it has been reported that FR results in increased levels of circulating corticosterone (Wan et al., 2003), a body homeostatic response to the need for enhanced gluconeogenesis. In contrast to detrimental stressors, such as chronic uncontrollable stress, which endanger neurons through glucocorticoid receptor activation, FR downregulates glucocorticoid receptors with maintenance of mineralocorticoid receptors in neurons which can act to prevent neuronal damage and death (Lee et al., 2000a,b).

Neurotrophic factors. As FR induce a mild stress response in brain cells this can result in the activation of compensating mechanisms, e.g. the upregulation of neurotrophic factors such as BDNF and glial cell line-derived neurotrophic factor (GDNF) as well as the aforementioned heat shock proteins (Bruce-Keller et al., 1999; Duan and Mattson, 1999; Duan et al., 2003; Maswood et al., 2004). It has been reported that FR induces the production of brainderived neurotrophic factor (BDNF) which was associated with increased hippocampal neurogenesis in rats and mice (Lee et al., 2002a,b). One of the primary neuroprotective mechanisms attributed to BDNF appears to be the ability of BDNF-mediated activation of its cognate TrkB receptor which then entrains stimulation of multiple signaling pathways. Prominent amongst these TrkB signaling pathways is the phosphatidyl inositol 3-kinase (PI3K)/protein kinase B (Akt) pathway that has been implicated in several of the FR protective mechanisms.

Ketone bodies. Dietary fasting is known to result in an increased production of ketone bodies, e.g. β-hydroxybutyrate, which can be used by the organism as an energy source in the face of limited glucose availability (Vazquez et al., 1985; Mitchell et al., 1995). IF dietary regimens can develop a two-fold increase in the fasting serum concentration of β-hydroxybutyrate compared with mice fed ad libitum (Anson et al., 2003).This shift to ketogenesis may play a direct role in the cytoprotective effects of IF, because it has been reported that rats fed a ketogenic diet exhibit increased resistance to seizures (Bough et al., 1999), and that ß-hydroxybutyrate itself can protect neurons in rodent models of Alzheimer's and Parkinson's diseases (Kashiwaya et al., 2000). Ketogenic diets, which promote a metabolic shift from glucose utilization to ketogen-

esis, are also prescribed for some patients with epilepsy (Gilbert et al., 2000) as this is prophylactic against the progressive excitotoxic neuronal damage and degradation that can occur if the condition is untreated.

Glucose/insulin signaling. During fasting or dietary restriction the primary alteration to the organism is the availability of glucose for oxidative respiration. The importance of glucose handling efficiency for healthy aging can be demonstrated by the fact that glucose levels in the blood, integrated over time, have been postulated to lead to high levels of non-enzymatic glycation, a form of protein damage. FR has been shown to specifically attenuate oxyradical production and damage (Weindruch and Sohal, 1997) and non-enzymatic glycation (Cefalu et al., 1995).

FR cause a reduction of insulin and glucose blood levels. A longitudinal study on male rats (Masoro et al., 1992) demonstrated that FR decreases the mean 24-h plasma glucose concentration by about 15 mg/dl and the insulin concentration by about 50%. FR animals utilized glucose at the same rate as did the rats fed ad libitum, despite the lower plasma glucose and markedly lower plasma insulin levels. Therefore, it is proposed that FR either increases glucose effectiveness or insulin responsiveness or both, and that the maintenance of low levels of glucose and insulin control the beneficial and life-extending actions of FR.

FR has also been found to reduce plasma glucose and insulin concentrations in fasting rhesus monkeys (Kemnitz et al., 1994). In addition, it can increase insulin sensitivity in rhesus and cynomolgus monkeys (Lane et al., 1995 and Cefalu et al., 1997). A major reason for this emphasis being placed on the insulinglucose control system in aging is the finding that loss-of-function mutations of the insulin signaling system result in life extension in three species: C. elegans (Kenyon et al., 1993; Wolkow et al., 2000), D. melanogaster (Clancy et al., 2001), and mice (Bluher et al., 2003).

Overall, from many experimental studies, FR seem to chronically reduce the circulating levels of insulin resulting in an eventual enhanced glucose mobilization and an enhanced insulin sensitivity, both of which serve to maintain a supply of glucose for the vital organs, central nervous system and gonads to support these critical organs in time of limited energy intake.

Cytokines. There is mounting evidence to suggest that inflammatory processes could be critically involved in the development of age-related pathologies such as those observed in Alzheimer's disease.

Recent findings suggest that IFN-γ is an important mediator of neuronal plasticity. Indeed, IFN-γ may enhance synaptogenesis, regulate synaptic plasticity and control neurogenesis (Improta et al., 1988; Vikman et al., 2001; Brask et al., 2004; Wong et al., 2004). It was recently reported that levels of IFN-γ are increased in circulating leukocytes of monkeys that had been maintained on a FR diet (Mascarucci et al., 2002). It has also been demonstrated that FR elevates the expression of IFN-γ in the hippocampus where it exerts an excitoprotective action (Lee et al., 2006).

Cytokines can also be produced by visceral organs outside the immune system and the central nervous system. Adipose tissue, which accumulates during aging and is specifically reduced upon FR regimens, can act as an endocrine organ, which produces trophic hormones active throughout the body (Bordone and Guarente, 2005), such as tumour necrosis factor-α (TNFα). TNFα has also been shown to trigger insulin resistance in animals (Feinstein et al., 1993). In vitro cell-culture studies have shown that TNFa renders cells insulin resistant through a downregulation of glucose transporter synthesis as well as through interference with insulin receptor signaling pathways (Stephens et al., 1997).

In vivo, the absence of the TNFα receptor significantly improves insulin sensitivity which mimics the insulin-related effects seen in FR animals. Interestingly, it has been shown that FR attenuates the age-related upregulation of nuclear factor (NF)-κB (Kim et al., 2000), which is a transcription factor that induces the expression of TNFα (Bordone and Guarente, 2005) in adipose tissue and the production of inflammatory cytokines in immune cells. Thus attenuation of TNF-α-induced insulin resistance may enhance the glucose utilization capacity of the organism, fending off the detrimental effects of excessive blood glucose that may occur in times of poor health and with advancing age.

Satiety and adipose generated hormones. Leptin and adiponectin are two hor-

mones that are typically associated with the feedback control of appetite and satiety. Both of these factors are produced by adipose tissue (Meier and Gressner, 2004) which is of course profoundly affected by FR. In addition to its role in satiety, leptin, released into the circulation, reduces the level of stress hormones (Barzilai and Gupta, 1999) and increases thyroid activity and thyroid-hormone levels which both result in increased energy expenditure (Legradi et al., 1997). As we have seen, FR regimens tend to upregulate stress hormones in a tolerable manner and in addition they can downregulate thyroid hormones, potentially through this attenuation of circulating leptin levels (Barzilai and Gupta, 1999). However leptin's role in mediating the beneficial effects of CR may be secondary to its satiety role as it has been demonstrated that mice that lack leptin demonstrate a reduced life-span, compared to ad libitum animals, and are obese (Allison et al., 2001).

Adiponectin has been shown to trigger increased insulin sensitivity (Meier and Gressner, 2004; Pajvani and Scherer, 2003) via upregulation of AMP-activated protein kinase (AMPK: Wu et al., 2003). This kinase regulates glucose and fat metabolism in muscle in response to energy limitation (Musi et al., 2001), and has been shown to protect neurons against metabolic stress (Culmsee et al., 2001). Importantly, adiponectin levels rise during FR, which suggests that this adipose-derived hormone might also have an important contributory role in the physiological shift to an enhanced insulin sensitivity in these animals (Combs et al., 2003).

Recent findings show that mice that have been genetically engineered to be lean live longer. Indeed, tissue-specific knockout of the insulin receptor in adipose cells prevents the tissue from storing fat, which gives rise to lean animals that live significantly longer than wild-type mice (Bluher et al., 2003). These data suggest that visceral adipose might be especially important in driving insulin resistance and pathogenesis (Bjorntorp, 1991).

Sirtuins. As lower organisms, e.g. yeast and nematode worms, possess a considerably shorter life-span than mammals they have proved useful for the discovery of the molecular determinants of healthy longevity. It has become apparent that amongst the multiple factors that have been identified that control life-span in these lower or-

ganisms, many of these also link the alteration of caloric intake to the increase in health-span.

One of the primary genetic determinants of replicative life-span to emerge from genetic studies in yeast is the silent information regulator 2 (SIR2). The SIR2 gene was denoted because it mediates a specific gene silencing action (Rine and Herskowitz, 1987). Inhibitory mutations of SIR2 can shorten life-span, and increased gene dosage of SIR2 extended life-span (Kaeberlein et al., 1999). The SIR2 ortholog in C. elegans was similarly shown to be a key determinant of the life-span in that organism (Tissenbaum and Guarente, 2001). As yeast and C. elegans diverged from a common ancestor about one billion years ago this may suggest that descendants of that ancestor, including mammals, will possess SIR2-related genes involved in regulating their life-span. As dietary regulation has also shown to be a powerful modulator of life-span it is reasonable to speculate that FR and SIR2 genes may converge to play an important role in these multiple and complex physiological pathways.

Mammalian homologues of the yeast SIR2 gene have subsequently been found and interestingly the SIR2 ortholog, SIRT1, may in part mediate a broad array of physiological effects that occur in animals on FR. The family of proteins discovered that are encoded for by the mammalian SIR2 homologues are collectively termed sirtuins. Several recent reports have shown increases in SIRT1 protein levels in response to food deprivation (Nemoto et al., 2004; Cohen et al., 2004). In addition SIR upregulation has been shown in response to cell stressors, such as high osmolarity (Lin et al., 2002a), thus the sirtuin family of proteins could be actively regulated by the mild, controllable stress induced by FR. Sirtuins possess a relatively rare enzymatic capacity as they are NAD-dependent histone deacetylases (Imai et al., 2000; Landry et al., 2000). The mammalian SIRT1 gene product enzyme can, in addition to histones, deacetylate many other substrates. In this regard, SIRT1 was recently shown to deacetylate and downregulate NF-κB (Yeung et al., 2004). It is intriguing to speculate that the upregulation of SIRT1 by FR contributes to the observed increase in insulin sensitivity and reduction in inflammation, potentially through the control of the NF-κ/TNFα pathways.

Lin et al. (2002a, 2004a) have proposed a molecular pathway for SIR2 activation that potentially connects alterations in caloric intake to life-span extension. Upon FR

there is an initial increase in oxygen consumption and respiration, at the expense of fermentative processes. Fermentation is a typical mechanism by which cells can generate ATP and also store excess energy in the form of ethanol when glucose is abundant. This metabolic shift triggers a concomitant reduction in NADH levels. NADH acts as a competitive inhibitor of SIR2, so its reduction during FR periods would be expected to upregulate the enzyme and thereby extend the organism's life-span in line with yeast and C. elegans studies. Consistent with this, ablation of mitochondrial electron transport blocked the effect of FR on life-span, and overexpressing NADH dehydrogenase, the enzyme that shunts electrons from NADH to the electron transport chain, increased the animal's life-span. Thus it appears that FRinduces a more efficient use of glucose via an increase in respiration.

In addition to this there is a transition in muscle cells from using glucose, which is to some extent, metabolized in ad libitum animals fermentatively (producing lactate), toward the use of fatty acids, which are oxidatively metabolized. This shift spares glucose for the brain, preventing neurodegeneration, and correlates with the characteristic enhancement of insulin sensitivity in muscle and liver seen in FR. Although the actions of sirtuins in the nervous system are only beginning to be explored, it has been reported that SIR2 (SIRT1 in mammals) activation through increased gene dosage or treatment with the sirtuin activator resveratrol can protect neurons against the pathogenic effects of polyglutamine-expanded huntingtin proteins in worm and mouse models of Huntington's disease (Parker et al., 2005).

Sirtuins also seem to play a role in mediating the effective role of adipose tissue in the physiological transference of the benefits of FR to the organism. One of the most important regulators of adipose tissue function is the peroxisome proliferator-activated transcription factor receptor gamma (PPARγ: Tontonoz et al., 1994). This receptor acts as a nuclear transcription factor that controls multiple genes connected to cell survival and responses to metabolic alterations. One PPARγ gene target, the aP2 gene, encodes a protein that assists fat storage. SIRT1 can act as a repressor of PPARγ, thereby downregulating genes such as the mouse aP2 gene (Picard et al., 2004). During fasting SIRT1 activation is followed by an enhanced binding to the aP2 promoter in adipose tissue. This causes a repression of aP2 gene expression causing

an eventual promotion of fat mobilization into the blood to aid the organism's energy balance. Therefore, according to Bordone and Guarente (2005), upon manipulation of caloric intake there is a reactionary activation of SIRT1 in adipose tissue, which acts to reduce fat stores and probably resets hormonal levels to change the pace of aging.

Peroxisome proliferator-activated receptor (PPAR) and co-factors. PPARs, as we have seen, are members of the nuclear hormone receptor subfamily of transcription factors. PPARs form functional heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate transcription of various genes. There are three known subtypes of PPARs, , δ and γ. These nuclear receptor transcription factors regulate genes involved in nutrient transport and metabolism as well as resistance to stress. PPARs themselves also recruit other proteins in addition to the RXR to mediate their complete function. One such protein is the peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1 (PGC-1). This coactivator has been shown to be closely regulated by dietary alteration in lower organisms and higher mammals. PGC-1 exists in two isoforms, α and β, and these isoforms have emerged as prominent regulators of the adaptive responses to caloric deprivation. PGC-1 regulates the ligand-dependent and -independent activation of a large number of nuclear receptors including the PPARs. There has been reported an age-dependent reduction in PGC-1α (Ling et al., 2004) which may exacerbate the aging process. However in mice and primates FR has been shown to reverse this age-dependent decrease in PGC-1α, PPAR and regulated genes (Weindruch et al., 2002; Kayo et al., 2001).

PGC-1α, the first PGC family member identified was characterized as a protein that interacts with the PPARγ to regulate brown fat differentiation during adaptation to cold stress (Puigserver et al., 1998). This cold stress may be regarded as analogous to the physiological and psychological stress induced by caloric restriction. During FR periods, when insulin levels are low, PGC-1α and PGC-1β gene expression is enhanced in rodents (Puigserver and Spiegelman, 2003; Herzig et al., 2001). PGC-1α was also induced in the livers of mice (Corton et al., 2004) and rats (Zhu et al., 2004) after longer term FR. PGC-1α and β can coordinately regulate genes involved in gluconeogenesis and fatty acid β-oxidation in a number of organs during fasting (Lin et al.,

2002b, 2004b; Kamei et al., 2003; Kressler et al., 2002). Both these processes are beneficial to the maintenance of a healthy energy balance in times of limited food. Hence through PPAR activation extra supplies of glucose can be mobilized and alternate energy sources can be exploited. As well as PGC regulation during fasting, PPARα is also upregulated by fasting in liver, small and large intestine, thymus (Escher et al., 2001), and pancreas (Gremlich et al., 2005). A large number of genes involved in fatty acid β-oxidation, known to be regulated by PPARα are also increased in expression in response to fasting. During periods of fasting PPARα knock-out mice exhibit an inability to regulate genes involved in fatty acid β- and γ-oxidation and ketogenesis in the liver, kidney and heart along with lack of control of blood levels of glucose or ketone bodies (Kroetz et al., 1998; Leone et al., 1999; Sugden et al., 2001).

Not only is the liver the energy-regulating core of mammals but it also represents one of the most significant stores of glycogen, nutrients and vitamins. One would therefore expect that there would be a critical link between alterations of caloric intake and resultant hepatic function. Thus it has been shown that FR protects the liver from a wide range of environmental stressors, many of which induce damage through circulating inflammatory mediators (Kim et al., 2002; Bokov et al., 2004). PPARα has been shown to regulate hepatic responses to diverse forms of stress. Mice pre-exposed to PPARα agonists exhibit decreased cellular damage, increased tissue repair, and decreased mortality after exposure to a number of physical and chemical hepatic stressors (Anderson et al., 2002; Wheeler et al., 2003). It appears that functional PPARs are crucial for the FR-mediated protection of the liver from damage induced by hepatotoxicants like thioacetamide. Specifically, it was demonstrated by Corton et al. (2004) that PPARα knock-out mice, in contrast to wild-type mice, were not protected from thioacetamide by FR regimens.

Lipid peroxide levels, associated with oxidative cell stress in the periphery and the central nervous system, are also significantly increased in aging. PPARα knock-out mice show a marked elevation in lipid peroxidation products compared to wild-type mice (Poynter and Daynes, 1998). Thus PPARα may influence aging through the regulation of multiple damage and repair processes after exposure to a plethora of endogenous or environmental stressors.

PGC-1 isoforms are transcriptionally or posttranslationally regulated in mammals by several signaling pathways implicated in the connection between FR and life-span extension. These include forkhead box other (FoxO) transcription factors (through an insulin/insulin-like growth factor-I -dependent pathway), glucagon-stimulated cellular AMP (cAMP) response element binding protein (CREB), stress-activated protein kinases (p38 and c-jun N-terminal kinase) and unsurprisingly SIRT1.

FoxO transcription factors. In mammals, insulin and IGF-I bind to either insulin or IGF-1 receptors activating multiple signaling pathways. With respect to the aging process and the amelioration of degenerative disorders it seems that the most important pathway entrained by insulin/IGF-1 is the canonical phosphatidylinositol 3-kinase (PI3K) and serinethreonine protein kinases (Akt-1/Akt-2/protein kinase B [PKB]) signaling cascade. In C. elegans, this pathway determines responses to longevity and environmental stress (Guarente and Kenyon, 2000). Mutations in C.elegans which inactivate the insulin/IGF-I pathway (as well as temperature and oxidative stresses), including Daf-2, the receptor for insulin/IGF-I or the PI3K ortholog Age-1, increase life-span. These effects require reversal of negative regulation of the stress resistance factor, Daf-16 (Libina et al., 2003). Daf-16 encodes a transcription factor containing a forkhead DNA binding domain. Overexpression of Daf-16 in worms (Henderson and Johnson, 2001) or an ortholog in flies (Giannakou et al., 2004) significantly extends their life-span. Daf-16 regulates the expression of an array of genes involved in xenobiotic metabolism and stress resistance (Murphy et al., 2003).

Mammalian homologs of Daf-16 fall into the family of FoxO factors. There are four main groups of mammalian FoxOs, FoxO1, FoxO3, FoxO4 and FoxO6. FoxO transcription factors belong to the larger Forkhead family of proteins, a family of transcriptional regulators characterized by the conserved forkhead box DNA-binding domain (Kaestner et al., 2000). These FoxO proteins control a wide array of genes that all are linked by a common mechanism in that they serve to control energy metabolim in the organism in response to environmental changes, e.g. restriction of available food. For example FoxOs control genes involved in glucose metabolism (glucose 6-phosphatase and phosphoenolpyruvate carboxylase: Nakae et al., 2001; Yeagley et al.,

2001); cell death (Fasligand), reactive oxygen species detoxification (catalse and manganese superoxide dismutase, Kops et al., 2002) and DNA repair (growth arrest and DNA damage-inducible protein 45 and damage-specific DNA-binding protein 1, Tran et al., 2002).

Insulin receptor stimulation, during caloric intake, leads to activation of the PI3K/Akt pathway and resultant phosphorylation of FoxOs in mammals. Phosphorylated FoxO factors are recognized by 14-3-3 proteins which facilitate their transport out of the nucleus, reducing their transcriptional activity. Thus upon FR there is a complex interplay between activation and inactivation of these FoxO factors. There are potentially beneficial effects of FoxO activation and inactivation depending upon the prevailing cellular conditions. Mammalian FoxO family members carry out functions that determine cell survival during times of stress including regulation of apoptosis, cell-cycle checkpoint control, and oxidative stress resistance (Coffer, 2003; Furukawa-Hibi et al., 2002). Activation of FoxO3 or FoxO4 leads to increases in cellcycle G1 arrest (van der Horst et al., 2004) and increases in apoptosis (Motta et al., 2004) presumably as a way to eliminate cells damaged by oxidative stress. Thus alterations in the capacity to activate the PI3K/Akt pathways can have dramatic effects upon cell survival and this process may be critical in transferring the positive effects of FR to the organism.

FR uncouples insulin/IGF-I signaling to FoxO factors by markedly reducing plasma IGF-I and insulin levels in rats (Sonntag et al., 1999). These decreases in circulating insulin/IGF-I levels result in decreased Akt phosphorylation in liver (Al-Regaiey et al., 2005) and decreased PI3K expression in muscle (Argentino et al., 2005). In addition there is a compensatory increase in the expression of FoxO family members by fasting (Imae et al., 2003; Furuyama et al., 2003) or FR (Al-Regaiey et al., 2005; Tsuchiya et al., 2004). Therefore, when insulin signaling is decreased, e.g. during FR there are not only increases in nuclear/cytoplasmic FoxO ratios but FoxO factor expression as well (Imae et al., 2003; Furuyama et al., 2003; Al-Regaiey et al., 2005; Tsuchiya et al., 2004). Overall, multiple studies have revealed that downregulation of insulin/IGF-I signaling results in increases in the activity of FoxO factors, that critically regulate cell survival mechanisms, and that these alterations are found consistently in

many diverse models of longevity among different species.

Many of the genes regulated by FoxOs are similarly regulated by the tumor suppressor p53, which has led to the speculation that these two genes may work in concert to prevent both deleterious aging and tumor growth. Consistent with this possibility, p53 and FoxO are both phosphorylated and acetylated in response to oxidative stress stimuli and UV radiations (Vousden and Lu, 2002; Brunet et al., 2004). In addition, both p53 and FoxOs bind to SIRT1 deacetylase (Luo et al., 2001; Vaziri et al., 2001). FoxO and p53 seem to be functionally linked as p53 can inhibit FoxO function by inducing serum and glucocorticoid induced kinase (SGK)-mediated phosphorylation of FoxO3 resulting in its relocation from the nucleus to the cytoplasm (You et al., 2004). FoxO3 has been found to prevent p53 from repressing SIRT1 gene expression. FoxO-induced repression of p53 appears to be mediated by the direct interaction between FoxO3 and p53 (Nemoto et al., 2004). That FoxO factors induce SIRT1 expression is consistent with the observation that SIRT1 expression is increased in rodent tissues when insulin and IGF-1 are lowered by CR (Cohen et al., 2004). In turn, SIRT1 itself can bind to and deacetylate p53 and FoxO transcription factors, controlling their activity. Mice harboring a mutation, which results in the activation of p53, display a significant reduction of life-span and exhibit signs of premature aging (Tyner et al., 2002). Interestingly, while activation of p53 in these mouse models reduces life-span, p53 activation still allows an increased resistance to cancer (Tyner et al., 2002), demonstrating that p53 causes tumor suppression at the expense of longevity.

One of the most important recent fields of caloric restriction study is the demonstration that FR may be able to prevent the generation of multiple forms of cancer itself. For example, in mice with genetically attenuated p53 levels FR increased the latency of spontaneous tumor development (mostly lymphomas) by approximately 75% (Hursting et al., 2001). It is therefore clear that there is a subtle and complicated relationship between these related factors that are linked together by changes in dietary energy intake.

In addition to negative regulation by insulin/IGF-1 signaling and p53, FoxO factors are regulated by the CREB binding protein (CBP) and a related protein, p300. Interestingly, cellular overexpression of CBP (Daitoku et al., 2004) or p300 (Fukuoka

et al., 2003) enhances the ability of FoxO factors to activate functional gene expression. SIRT1 again seems to play a central role in adaptive changes to energy regulation as it can reverse the negative regulation of FoxO family members by CBP. Like PGC-1, SIRT1 levels are increased during FR in rat liver and are negatively regulated by insulin and IGF-I (Cohen et al., 2004). Additionally, the related family member SIRT3, a mitochondrial protein, exhibits increased expression in white and brown fat upon CR (Shi et al., 2005).

FoxOs seem to exist at a nexus between mechanisms that connect cellular stress responses to eventual survival mechanisms. For instance the stress-related protein kinase cJun N-terminal kinase 1 (JNK-1), which serves as a molecular sensor for various stressors actively can control FoxO transcriptional action. In C. elegans, JNK-1 directly interacts with and phosphorylates the FoxO homologue Daf-16, and in response to heat stress, JNK-1 promotes the translocation of Daf-16 into the nucleus. Overexpression of JNK-1 in C. elegans leads to increases in lifespan and increased survival after heat stress (Oh et al., 2005). In D. melanogaster as well, mild activation of JNK leads to increased stress tolerance and longevity (Wang et al., 2003) dependent on an intact FoxO (Wang et al., 2005).

In conclusion it seems that FoxO transcription factors are promising candidates to serve as molecular links between dietary modifications and longevity. In conditions such as FR where the circulating levels of insulin/IGF-1 are attenuated to improve euglycemia, FoxO nuclear translocation results in the upregulation of a series of target genes that promote cell cycle arrest, stress resistance, and apoptosis. External stressful stimuli also trigger the relocalization of FoxO factors into the nucleus, thus allowing an adaptive response to stress stimuli. Consistent with the notion that stress resistance is highly coupled with life-span extension, activation of FoxO transcription factors in worms and flies increases longevity. FoxO proteins translate environmental stimuli, including the stress induced by caloric restriction into changes in gene expression programs that may coordinate organismal healthy aging and eventual longevity.

MATERIALS AND METHODS

Animals treatment

A total of 90 Long -Evans hooded rats aged between P60 and P90 were used in this study. Animals were housed two/three per cage at 20-22 °C under a 12-h light/dark cycle and had ad libitum access to water. At P60 animals were randomly divided into two nutritional groups: an ad libitum group (CTL) with unlimited access to food, and a food restricted (FR) group consisting of animals with access to food only on alternate days. The total duration of the dietary regimen was 4 weeks for all the animals. Once a week the animals were weighted and in the last ten days of dietetic regimen food intke was measured.

The animals tested for visual cortex plasticity were monocular deprived through eyelid suturing after three weeks of food restriction. Eyelid closure was inspected daily until complete cicatrization. One week after monocular deprivation (MD) the animals were subjected to in vivo electrophysiology to test ocular dominance (OD) shift.

In a second group of animals MD was performed through eyelid suturing at postnatal day (P) 21. Once adult (P60) they were divided in FR and CTL and, after two weeks of dietetic regimen, reverse suture (RS) was performed under anesthesia. The long-term deprived eye was re-opened using thin scissors, while the other eye was sutured. Great care was taken during the first week after RS to prevent inflammation or infection in the previously deprived eye through topical application of antibiotic and cortisone. After two weeks from RS the animals were subjected to electrophysiological recordings to study the recovery of binocularity and visual acuity.

Corticosterone measurement

Blood samples were collected by decapitation and added with EDTA, centrifuged 4000 rpm, 4°C, and stored at -80° until use. Rat corticosterone was measured with EIA test (Rat Corticosterone EIA, Diagnostic Systems Laboratories, Inc.) according the protocol included.

It has been widely demonstrated that the circulating plasma glucocorticoid hormone (corticosterone in rodents, cortisol in humans) levels are subjected to circadian variation. In the rat the circadian rhythm is characterized by low levels of plasma corticosterone in the early morning and higher levels towards the evening, the active phase of this nocturnal animal. In contrast, in humans, who sleep during the night, glucocorticoid levels peak in the early morning (Droste et al., 2008). For these reasons we have measured the levels of corticosterone always at the same time of the day, at the beginning of the nocturnal phase, in order to analyze the relative quantity of blood hormone at the peak of its secretion.

Briefly, 25 μl of Standards, controls provided with the kit, FR and controls diluted 1:10 blood samples were loaded in duplicate on microtiter wells pre treated with goat anti-rabbit IgG, added with anti Rat corticosterone enzyme conjugate solution and incubated for 24h at RT. After extensive washing, TMB substrate was added to each well and incubated 30min. The reaction was stopped with addition of HCl 1M and absorbance measured on a Bio Rad 450 microtiters reader at 450 nm after 15 min. The corticosterone levels were calculated from the standard curve obtained with a 4 parameters curve fit of the [Mean Absorbance/(Mean Absorbance of blanks)*100] vs (Corticosterone ng/ml).

Elevated plus maze test

The EPM was made of wood and consisted of two open arms (50x10 cm), opposite to two closed arms, crossed with two enclosed arms, with an open roof (50x10x40 cm). The Maze was elevated 50 cm from the ground floor and it was situated in a quiet, dimly lit room. A high sensitive video camera was installed on the roof of the experimental room and all sessions were recorded on a digital format.

The behavioral test was made in the morning after one day of fasting, when levels of corticosterone were higher, and lasted 5 minutes. At the end of testing each animal, the maze was cleaned with a 10% alcohol solution. Time spent in open arms was then measured for each individual.

Locomotor activity

A computerized Opto M3 Animal Activity Monitoring System (Columbus Instruments, Columbus, OH) was used to monitor locomotor activity in rats. Each activity cage consisted of a box (20 × 20 × 20 cm) surrounded by horizontal and vertical infrared sensor beams. The locomotor activity of FR (n=2) and CTL (n=2) rats was recorded for 3 hours during the light period and 3 hours during the dark period in the animal facility room in which the rats had been housed. The measurements were done for 2 consecutive days (day of feeding and day of fasting for FR animls) every 4 days during the whole period of diet.

In vivo electrophysiology

At the end of the dietary regimen the animals were anesthetized with urethane (0.7 ml/hg; 20% solution in saline; Sigma) by i.p. injection and placed in a stereotaxic frame. Additional doses of urethane were used to keep the anesthesia level stable throughout the experiment. Body temperature was continuously monitored and maintained at 37°C by a thermostated electric blanket during the experiment. An ECG was continuously monitored. A hole was drilled in the skull, corresponding to the binocular portion of the primary visual cortex (binocular area Oc1B) contralateral to the deprived eye. After exposure of the brain surface, the dura was removed, and a micropipette (2MΩ) filled with NaCl (3M) was inserted into the cortex 5 mm from intersection between sagittal- and lambdoid-sutures. Both eyes were fixed and kept open by means of adjustable metal rings surrounding the external portion of the eye bulb. We measured visual acuity through both eyes using visual evoked potentials (VEPs). During recording through one eye, the other was covered by a black adhesive tape. To record VEPs, the electrode was advanced at a depth of 100 or 400 ìm within the cortex. At these depths, VEPs had their maximal amplitude. Signals were

band-pass-filtered (0.1100 Hz), amplified, and fed to a computer for analysis. Briefly, at least 128 events were averaged in synchrony with the stimulus contrast reversal. Transient VEPs in response to abrupt contrast reversal (0.5 Hz) were evaluated in the time domain by measuring the peak-to-baseline amplitude and peak latency of the major negative component. Visual stimuli were horizontal sinusoidal gratings of different spatial frequencies, generated by a VSG2/2 card running custom software and presented on a monitor (20 x 22 cm; luminance 15 cd/m2) positioned 20 cm from the rats eyes and centred on the previously determined receptive fields. Visual acuity was obtained by extrapolation to zero amplitude of the linear regression through the last four to five data points in a curve where VEP amplitude is plotted against log spatial frequency. Binocularity (ocular dominance) was assessed calculating the contralateral to ipsilateral (C/I) VEP ratio, i.e. the ratio of VEP amplitudes recorded by stimulating the eye respectively contralateral and ipsilateral to the visual cortex where recording was performed.

Visual cortex LTP

Brains from adult FR rats or controls were removed and immersed in ice-cold cutting solution containing (in mM): NaCl, 3.1 KCl, 1.0 K_2HPO4, 4.0 NaHCO3, 2.0 $MgCl_2$, 1.0 $CaCl_2$, 10 HEPES, 1.0 ascorbic acid, 0.5 myo-Inositol, 2.0 pyruvic acid, and 1.0 kynurenate, pH 7.3. Slices (0.35mm thick) of visual cortex were obtained using a Leica (Nussloch, Germany) vibratome. Slices were perfused at a rate of 2 ml/min with 32°C oxygenated recording solution. The recording solution was composed as the cutting solution with the following differences (in mM): 130 NaCl, 5.0 dextrose, 1.0 $MgCl_2$,2.0 $CaCl_2$, 0.01 glycine, no kynurenate. Electrical stimulation (100 μsec duration) was delivered with a bipolar concentric stimulating electrode (FHC, St. Bowdoinham, ME) placed at the border of the white matter and layer VI. Field potentials in layer III were recorded by a micropipette (13MΩ) filled with recording solution. Baseline responses were obtained every 30 sec with a stimulation intensity that yielded a half-maximal response. After achievement of a 20 min stable baseline (field potential amplitude within 15% of change and with no evident increasing or decreasing trends), θ-burst stimulation (TBS) was delivered.

Hippocampal LTP

Brains from controls and FR adult rats were removed. Transverse hippocampal slices (400 μm) were cut and single slices were continuously perfused at 32°C with cutting solution containing (in mM): NaCl, 3.1 KCl, 1.0 K_2HPO4, 4.0 $NaHCO_3$, 2.0 $MgCl_2$, 1.0 $CaCl_2$, 10 HEPES, 1.0 ascorbic acid, 0.5 myo-Inositol, 2.0 pyruvic acid and 1.0 kynurenate, pH 7.3. fEPSPs were recorded in stratum radiatum of CA1 hippocampal region. A concentric bipolar stainless steel electrode was placed in the stratum radiatum for stimulating the Schaeffer collateral afferents (0.1 ms pulse duration). Test stimuli were applied at a stimulus intensity that elicited an fEPSP amplitude that was about 50% of maximum. Long-term potentiation (LTP) was induced by a high-frequency stimulation (HFS) consisting of four 100-Hz trains applied with an interval of 5 minutes; stimulus width was 0.2 ms during the trains.

In vivo brain microdialysis

To perform brain microdialysis, adult rats were anesthetized and stereotaxically implanted with stainless steel guide shafts above the binocular visual cortex (binocular area Oc1B), immediately after RS, at coordinates: 7.3 mm posterior to bregma, 4.4 mm lateral to the midsagittal suture and 1 mm ventral to the skull. After four weeks of differential rearing (under FR conditions or not), *in vivo* sampling of dialysates was performed inserting a microdialysis probe into the guide shaft previously implanted. Briefly, the probe was made of concentric fused-silica polyimide covered capillary tube into a 26 gauge stainless steel tube with a 1 mm long tip of exposed cellulose membrane (6000 MW cutoff). It was connected to a dialysis system pumping an artificial CSF (142 mM NaCl, 3.9 mM KCl, 1.2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.35 mM Na_2HPO_4, pH 7.4) at a flow rate of 1 μl/min. The probe protruded 1 mm from the tip of the guide shaft. Six hours after insertion of the probe (stabilization period), sampling was carried out. Six samples (20 μl each) were collected every 20 min along 2 hours for each freely moving FR (n=7) and control animal (n=5).

High Performance Liquid Chromatography (HPLC)

Analysis of γ-aminobutyric acid (GABA) and glutamate (GLU) basal levels from microdialysates was performed using High Performance Liquid Chromatography (HPLC) coupled to a fluorimetric detection system. A sample automatic derivatization (Waters 2690 Alliance) with o-phtalaldehyde was followed. Resolution was obtained through a C18 reverse phase chromatographic column coupled to the fluorimetric detection (Waters 474; excitation wavelength 350nm, emission wavelength recorder 450nm). Buffer and gradient program was as follows: by definition, solvent A: 0.1M Sodium Acetate pH 5.8/methanol 20/80; solvent B: 0.1M Sodium Acetate pH 5.8/methanol 80/20; solvent C: 0.1M Sodium Acetate pH 6.0/methanol 80/20. Concerning the gradient program, initial isocratic step 5% A, 95% C from 0 to 5 min; 15% A, 85% B from 4 to 5 min and then isocratic until 9 min; 22% A, 66% B until 14.5 min and then 34% A, 66% B until 17 min; 5% A, 95% C until 19 min and then isocratic until 23 min. Flow rate was 0.9 ml min1. Homoserine was used as internal standard and aminoacid concentrations were calculated from a linear standard curve built upon known concentrations of injected aminoacids. Area of the peaks were used to make comparisons (Waters Millenium 32).

Western blotting

A total of 16 rats were killed by decapitation, and brains were removed rapidly and frozen on dry ice. Cortices and hippocampi from each animal were then homogenized in a hypotonic lysis buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonylfluoride, 10 μg/ml Aprotinin, 10 μg/ml Leupeptin (Sigma, Italy), and 1% Igepal CA-630.

BDNF. Protein concentration was determined by Biorad assay (Biorad, Italy). Each sample was boiled, and 25 μg/lane was loaded into 12% acrylamide gels using the Precast Gel System (Biorad, Italy). Samples were blotted onto nitrocellulose membrane (Amersham, Bucks, UK). The membrane was cut to separate BDNF from β-tubulin with respect to their molecular weight and then blocked in 4% nonfat dry milk (or 4%

BSA for β-tubulin) in Tris-buffered saline for 1 hr. Then membranes were probed with anti-BDNF polyclonal antibody (1:200; N-20, Santa Cruz) or anti-β-tubulin (1:6000; Sigma). Both antibodies were diluted in TTBS with 2% milk or 2% BSA and incubated overnight at 4°C. Blots were then rinsed for 20 min in TTBS, incubated in HRP-conjugated anti-mouse or anti-rabbit (1:3000 Biorad, Italy, in 2% milk or 2% BSA and TTBS), rinsed, incubated in enhanced chemiluminescent substrate (Biorad, Italy), and exposed to film (Hyperfilm, Amersham Biosciences, Europe).

Films were digitalized with a Epson Perfection 3200 Photo scanner and band optical densityes (OD) were analyzed with ImageJ software (freewaresoftware ver. 1.41o). The amount of BDNF protein was evaluated measuring the OD of the BDNF band at 14kD and dividing it by the OD of β-tubulin band on the same filter at 50kD. Data from each sample were normalized to the controls, run on the same gel, and summarized results presented as a percentage of control values (mean±SEM).

Histones. Histones were extracted from the nuclear fraction by the addition of five volumes of 0.2 M HCl and 10% glycerol, and the insoluble fraction was pelleted by centrifugation (18,000 × g; 30 min; 4°C). Histones in the acid supernatant were precipitated with ten volumes of ice-cold acetone followed by centrifugation (18,000 × g; 30 min; 4°C). The histone pellet was then resuspended in 9 M urea. Protein concentration was determined by Biorad assay (Biorad, Italy). Each sample was boiled, and 30 μg/lane was loaded into 12% acrylamide gels using the Precast Gel System (Biorad, Italy). Samples were blotted onto nitrocellulose membrane (Amersham, Bucks, UK), blocked in 4% nonfat dry milk in Tris-buffered saline for 1 hr, and then probed with antibodies for AcH3 and H3 (Upstate, NY). All antibodies were diluted in TTBS and 2% milk or 2% BSA and incubated overnight at 4°C. Blots were then rinsed for 20 min in TTBS, incubated in HRP-conjugated anti-mouse or anti-rabbit (1:3000 Biorad, Italy, in 2% milk or 2% BSA and TTBS), rinsed, incubated in enhanced chemiluminescent substrate (Biorad, Italy), and exposed to film (Hyperfilm, Amersham Biosciences, Europe). Films were scanned, and densitometry was analyzed through ImageJ software. To minimize variability, each sample was loaded in parallel in two lanes and two gels were run simultaneously on the same apparatus. For each gel, the corresponding filters obtained after blotting were cut in two in order to obtain in each filter a complete

series of samples. One of the two filters was reacted with an antibody for the modified protein (AcH3) and the other with an antibody insensitive to the target protein modifications (H3). The densitometric quantification of the band corresponding to the modified protein was then normalized to the value obtained for the total amount of protein from the same gel. Each sample was analyzed four times using this procedure.

Immunohistochemistry

Animals perfusion (n=6 for both FR and CTL rats) was performed with 4% paraformaldehyde in phosphate buffer and brains were post-fixed overnight before being immersed in 30% sucrose. Fifty Êm coronal sections from the occipital cortex were cut on a sledge microtome and collected in PBS.

WFA staining. Free-floating sections were incubated for 60 minutes in a blocking solution composed of 3% BSA, 0.1% Triton X-100, in PBS, pH 7.4. Sections were incubated overnight at 4°C in a solution of biotin-conjugated lectin Wisteria Floribunda (WFA) (Sigma, 10 μg/ml). WFA was stained with a 1h incubation in cy3-conjugated extravidin (10 μg/ml, Sigma) and then mounted on slides with Vectashield. Sections of the two different experimental groups were reacted together with the same immunohistochemical procedure.

BDNF staining. For BDNF immunostaining, we used the same brains reacted for WFA. After a blocking step, sections were incubated overnight in chicken polyclonal anti-BDNF antibody (1:400, Promega). Biotinylated donkey anti-chicken was used as secondary antibody (2hrs incubation) and sections were stained with a 1h incubation in cy3-conjugated extravidin then mounted on slides with Vectashield. Immunostaining was performed for CTL and FR brain sections in parallel within the same experimental set. Images were acquired, blind to the treatment, with the same method described for WFA staining. An average of 5 fields were acquired for each animal.

Acquisition and quantification of images. Images from the binocular primary visual cortex were acquired at 20× magnification at 1024×1024 pixel resolution using a laser-scanning confocal microscope (Olympus, Japan). Settings for laser intensity, gain, offset and pinhole were optimized initially and held constant through the experiment. For each animal, at least 5 slices were acquired and the section with the

highest signal were acquired. Cells positive for each marker were counted manually with the MetaMorph software (Universal Imaging Corp., USA) and their density was calculated. All analyses were done using a blind procedure.

GAD65 staining. Free floating sections were incubated for 1 hour in a blocking solution (containing 10% BSA, 0,3% Triton X-100 in PBS). Then section were incubated overnight with monoclonal antibody anti GAD65 (Chemicon, MAB351, 1:500, in 1% BSA, 0,2% Triton) and revealed with biotinylated secondary antibody goat anti-mouse IgG (1:200, Vector Laboratories, Burlingame, CA) followed by incubation in cy3-conjugated extravidin (1:500, Sigma). Sections were then mounted on slides with Vectashield.

Images were acquired at 60×(N.A. = 1,40, field 105×105 μm acquired at 512×512 pixels). Settings for laser intensity, gain, offset and pinhole were optimized initially and held constant through the study. During image collection, confocal settings were regulated so that the full range of pixel intensities (0-255) was used, with very little saturation at either end of intensity range. For each animal at least four sections were analyzed. For each section, we imaged three field taken from layer II/III of the primary visual cortex. In each field, a stack the three focal planes, spaced 1 μm each one, with the highest signal were acquired. Images were superimposed with the MetaMorph software (Universal Imaging Corp., USA). Perisomatic GAD65 signals (e.g. puncta-ring) from at least three target neurons were outlined for each image and GAD65 signal intensity was calculated. For each neuron, signal intensity were divided by the background labelling in the cell soma. A total sample of 4-5 neurons were analyzed for each cortex. All images acquisition and analysis were carried out in blind.

RESULTS

Effects of food restriction on food intake and body weight

To first analyze the effects of our short-term protocol of FR on the general physiology of the animals we started measuring the total food intake during the period of dietetic regimen. The FR group received food on alternate days, with maintenance of the composition of the diet in terms of vitamins, minerals, proteins etc.. After one month of dietary regimen the FR group showed a reduction of about 22% in the daily food assumption (Fig.2a). This is not a great decrease with respect to other kind of protocols in which the animals receive a daily amount of food that is between the 30 and the 60% of the normal consumption. The FR protocol had a significant effect also on the body weight of the animals kept under dietary regimen. Indeed, the body weight of food restricted rats was always lower than controls, starting from the first week of diet. At the end of the 4th week of FR, mean body weight of restricted rats decreased of about 10% with respect to controls (Fig.2b).

Corticosterone levels in the blood of food restricted rats

Most studies on FR have shown that a reduction in the caloric intake is accompanied by an increase in the blood content of corticosterone (Stamp et al., 2008; Patel and Finch 2002), a body homeostatic response to the need for enhanced gluconeogenesis. Moreover, it has been widely demonstrated that the circulating plasma glucocorticoid hormone (corticosterone in rodents, cortisol in humans) levels are subjected to circadian variation. In the rat the circadian rhythm is characterized by low levels of plasma corticosterone in the early morning and higher levels towards the evening, the active phase of this nocturnal animal (Droste et al., 2008). For this reason I have measured

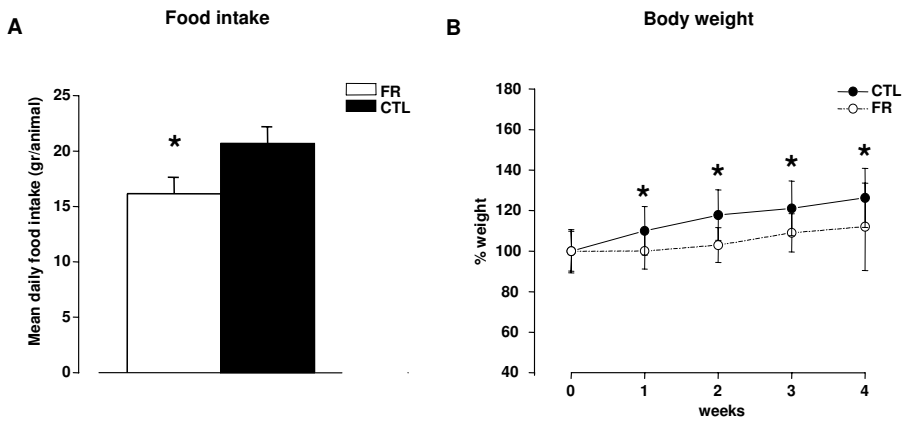

Figure 2: **Measure of Food intake and body weight in FR rats**. A) Food intake was measured in the last 10 days of diet. Food intake of FR rats (n=6; mean daily food intake 16.15±1.47gr) was significantly lower with respect to controls (n=6; mean daily food intake 20.67±1.52gr; Two Way ANOVA $p<0.001$). B) Body weight measured during the whole period of dietetic regimen showed a significant reduction in the FR animals since the first week of diet. At the end of treatment mean body weight of FR rats was 112.06%±21.56% of the initial value, while mean body weight of controls was 126.31%±14.55% of their initial value. The difference between the two groups was always statistically significant (n FR=6; n CTL=6; Holm-Sidak test on rank $p<0.001$). Error bars represent SEM; asterisks indicate statistical significance.

the levels of corticosterone in FR rats always at the same time of the day at the beginning of the nocturnal phase, in order to analyze the relative quantity of blood hormone at the peak of its secretion.

In FR rats I observed almost a 100% increase in the blood content of corticosterone. Noteworthy, this increase was present only at the end of a fasting day, while corticosterone levels returned to normality after one day of feeding (Fig.3a). This result confirms that the protocol of FR is only a mild stressor being the increase of serum corticosterone levels not continuous and sustained during time (Rattan, 2008; Masoro 2007). Moreover, FR rats did not show any increase in mean daily locomotor activity; on the contrary, they seem to have an activity even lower with respect to controls (Fig.3b) and, when subjected to the elevated plus maze test, they showed a behaviour very similar to the control group (Fig.3c).

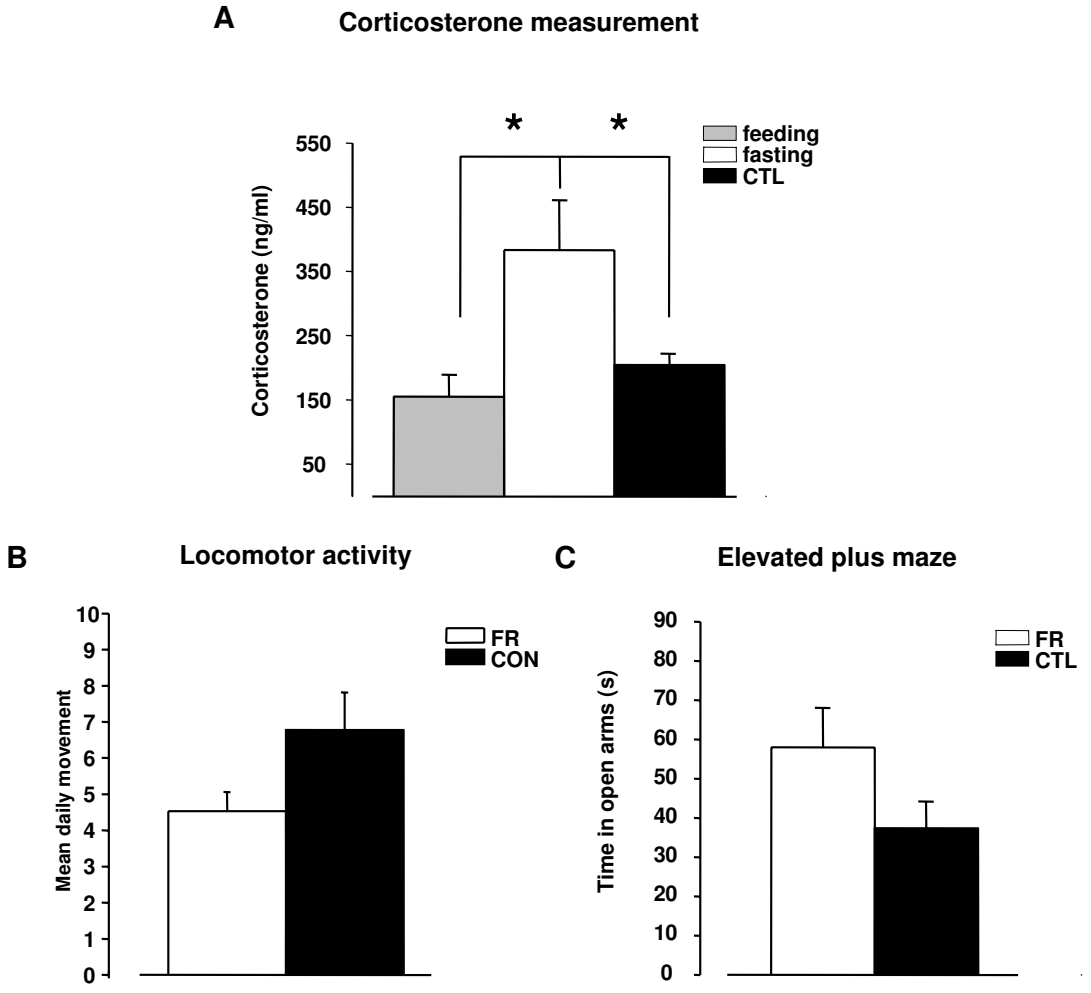

Figure 3: **Measure of corticosterone and stress behaviour in FR rats.**. A) Serum content of corticosterone in FR animals and controls. In FR rats (n=6) corticosterone levels were significantly higher with respect to controls (n=10) at the end of a fasting day (383.75±77.5 ng/ml vs. 205.09±17.05 ng/ml; t-test p=0.026), while they returned to normality after one day of feeding (n=7; 155.10±34.25 ng/ml; feeding vs. controls t-test p=0.173). B) Mean daily locomotor activity was lower in FR rats with respect to controls C) When tested in the elevated plus maze test FR rats (n=6) spent the same time in open arms with respect to controls (n=6; 58±10 sec vs. 37±7 sec; t-test p=0.123). Error bars represent SEM; asterisks indicate statistical significance.

Restoration of OD plasticity following FR in adult rats

To assess whether reducing the caloric intake could restore visual cortex plasticity in adulthood I used two classical models of visual system plasticity: 1) the ocular dominance (OD) shift of cortical neurons after monocular deprivation (MD) and 2) the recovery from amblyopia after reverse suture (RS). The effectiveness of these two manipulations in the rat are restricted to the CP.

To test whether 7 days of MD were effective in shifting the OD distribution (binocularity) of adult FR rats, the animals were monocularly deprived in the last week of the dietetic regimen and OD shift was assessed. I used VEP recordings to establish the ratio between the response of cortical neurons to the stimulation of either eye, a validated method for binocularity measurement (Porciatti et al., 1999). The contra/ipsi (C/I) VEP ratio is in the range of 2-3 in adult normal animals, while its value is strongly reduced (about 1) when rats are subjected to MD during the critical period, reflecting the occurred OD shift.

I have shown that one week of MD is effective in shifting the OD distribution of FR rats being the VEP ratio mean value of this group typical of animals deprived during the critical period (C/I VEP ratio=1.29±0.21; n=8; t-test, $p<0.001$). On the contrary, the VEP ratio of MD controls is not different from that of normal adult rats (C/I VEP ratio=2.81±0.21; n=5; Fig.4a). I also measured visual acuity (VA) by VEP recordings in the visual cortex contralateral to the closed eye and found that VA of the deprived eye was significantly reduced in FR rats (0.86±0.05 vs 1.04±0.04; n=6; paired t-test p=0.027; Fig.4b).

I next evaluated whether this potential for plasticity could be useful to restore normal visual functions in amblyopic animals. Rats rendered amblyopic by long-term MD starting from P21 were subjected to FR protocol in adulthood (P60) and reverse suture was performed during the last two weeks of diet. In adult FR rats binocularity (C/I VEP ratio=2.08±0.12; n=7; t-test, $p<0.001$) and VA (1.01±0.05 vs. 1.02±0.07; n=5; paired t-test p=0.851) of the amblyopic eye exhibited a complete rescue. In contrast I did not observe any sign of recovery in control animals (C/I VEP ratio=1.21±0.14; n=6; VA: 0.72±0.03 vs. 1.03±0.02; n=4; paired t-test p=0.004; Fig.5a,b).

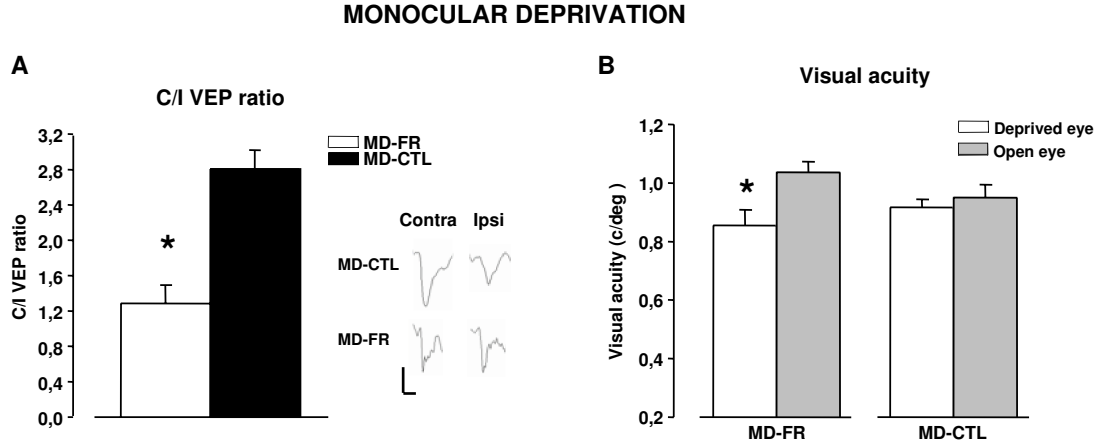

Figure 4: **FR reactivates visual cortical plasticity in adulthood.**. A) MD did not affect OD in adult control animals (n=5; C/I VEP ratio=2.81±0.21) whereas FR rats showed a significant decrease of C/I VEP ratio (n=8; 1.29±0.21; t-test, $p<0.001$) indicating a full OD shift. Typical VEP responses to the stimulation of either eye are also shown. Calibration bars: 50μV, 100msec B) VA of the deprived eye was reduced in FR rats (n=6; 0.86±0.05 vs 1.04±0.04; paired t-test p=0.027) but not in controls (n=6; 0.92±0.03 vs 0.95±0.04; paired t-test p=0.385). Error bars represent SEM; asterisks indicate statistical significance.

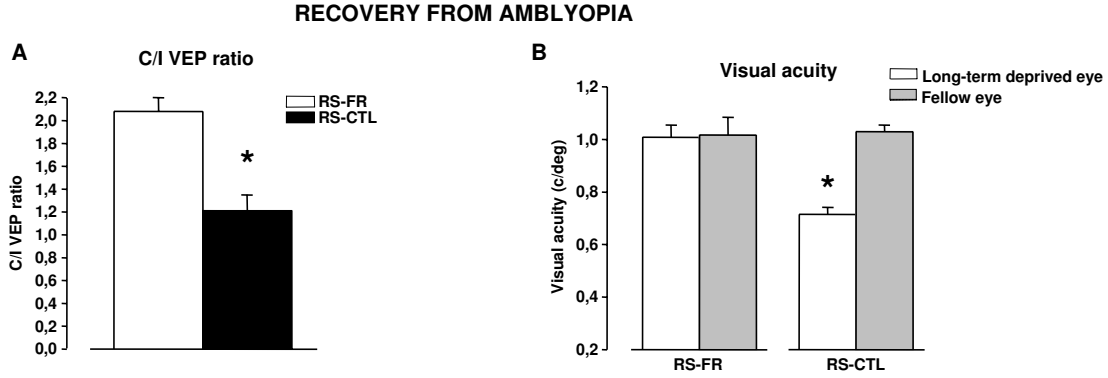

Figure 5: **FR promotes recovery from amblyopia in adulthood**. A) OD in amblyopic rats was recovered in FR rats (n=7; 2.08±0.12) but not in controls (n=6; 1.21±0.14; t-test, $p<0.001$) and was in the range of adult normal values. B) VA of the long-term deprived eye was not different from that of the fellow eye in FR rats (n=5; 1.01±0.05 vs. 1.02±0.07; paired t-test p=0.851) while it remained significantly lower in controls (n=4; 0.72±0.03 vs. 1.03±0.02; paired t-test p=0.004). Error bars represent SEM; asterisks indicate statistical significance.

FR is accompanied by a decrease in GABA release and GAD65 expression in the visual cortex

Inhibition is a common link between experimental protocols that increase adult plasticity. Indeed, there is evidence that the inhibitory tone is essential for the time course of critical period in the visual cortex (Hensch et al., 1998) and it has been demonstrated that strategies that decrease inhibition reactivate developmental levels of plasticity in the adult brain (Ehninger et al., 2008).

To investigate the inhibitory system function in FR rats, we analysed the release of GABA in their visual cortex by means of in vivo brain microdyalisis. Extracellular basal levels of GABA were significantly reduced in FR rats with respect to controls (pmol/μL=0.51$\pm$0.12 vs. 2.94$\pm$0.33; n FR=7; n controls=6; t-test $p<0.001$; Fig.6a,b) while no difference in glutammate levels was detected (Fig.6c,d).

The decrease of GABA release was correlated with reduced expression of GAD65 (the enzyme responsible for GABA synthesis at axon terminals) as assessed by means of quantitative immunohistochemistry (t-test p=0.001; Fig.7).

FR restores WM-evoked layer III LTP in the visual cortex and enhances CA3-CA1 LTP in the hippocampus of adult rats

The development of inhibition has been proposed to reduce the induction of plasticity in the visual cortex by acting as a gate which filters the level and pattern of activity that layer IV, the major thalamo-recipient layer, is able to relay to supragranular layers (Huang et al., 1999; Trachtenberg et al., 2000; Rozas et al., 2001). Indeed, it has been demonstrated that the induction of LTP in the layer II-III of the cortex through stimulation of the white matter (WM-LTP) declines with age and acute application of GABA receptor antagonists on visual cortical slices at the end of the critical period increases the probability of LTP induction (Kirkwood et al., 1995). To test whether our FR protocol which is able to restore experience dependent plasticity to the adult visual cortex also regulates activity dependent synaptic plasticity I assessed whether WM-evoked layer III LTP is inducible in slices from the cortex of adult FR rats.

Adult animals were subjected to FR for a month and at the end of this pe-

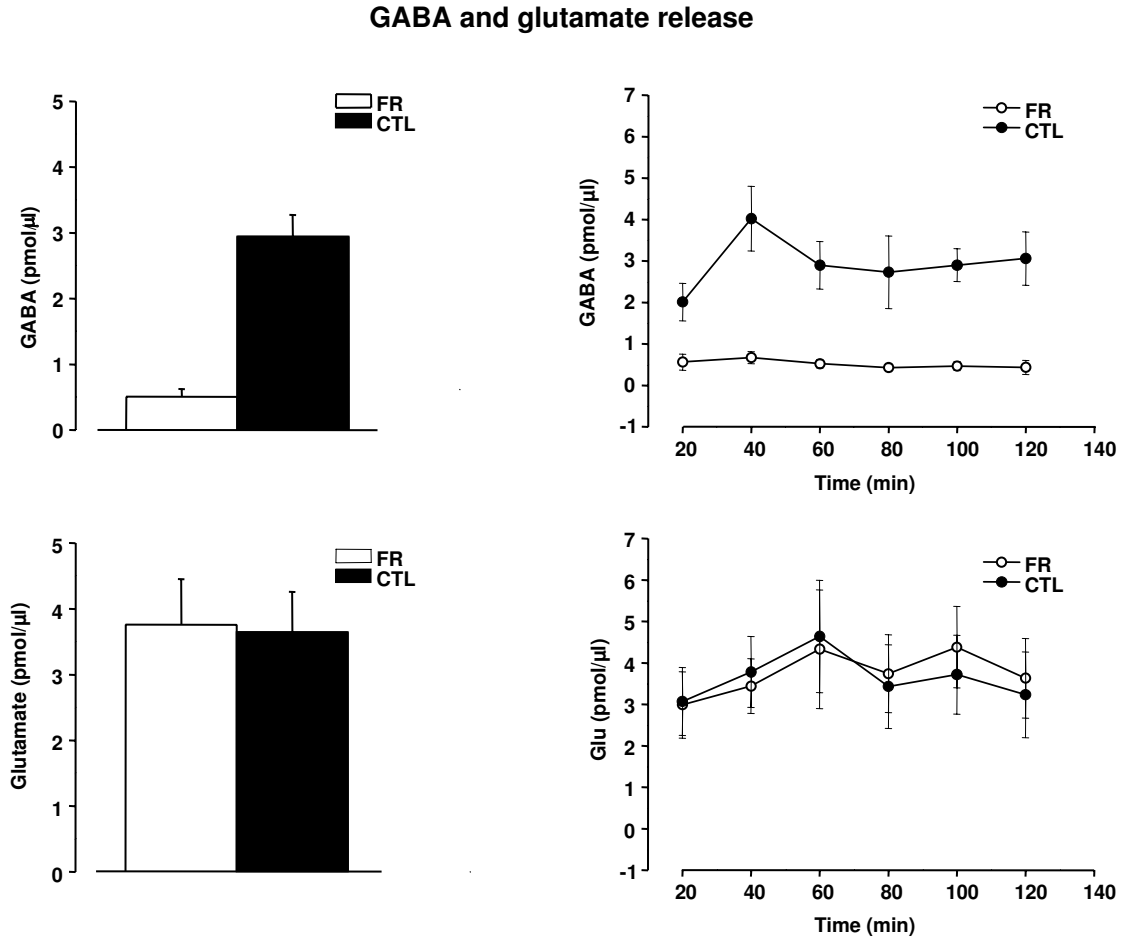

Figure 6: **FR is accompanied by reduced GABAergic inhibition**. In vivo brain microdyalisis revealed a significant decrease of GABA release in the visual cortex of FR rats (n=7; pmol/μL=0.51±0.12; t-test p<0.001) with respect to controls (n=5; pmol/μL=2.94±0.33). No difference was found in basal release of glutamate between FR rats (n=7; pmol/μL=3.75±0.69) and controls (n=5; pmol/μL=3.64±0.61; t-test p=0.909). Basal release of GABA and glutamate during the 2 hours of sample collection is also shown. Error bars represent SEM; asterisks indicate statistical significance.

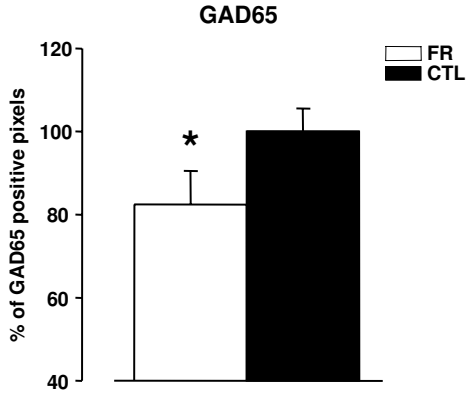

Figure 7: GAD65 expression quantified through immunohistochemistry was significantly lower in the visual cortex of FR rats (n=6) than in controls (n=6; t-test p=0.001).

riod WM-evoked layer III LTP was assessed. Slices prepared from the visual cortex of control adult animals served as control group. As expected, LTP induction was absent in the controls, while visual cortical slices taken form FR rats showed a robust potentiation of Field Potential (FP) amplitudes after WM stimulation (n FR=7 slices, 5 animals; n controls=7 slices, 5 animals; Two way ANOVA FR vs. controls $p<0.001$; Fig.8a).

The dietary regimen has a general impact on the whole organims and it is very likely that the effects of FR are not confined to the visual cortex. To test this hypothesis, I measured LTP expression induced by Shaffer collateral stimulation in the CA1 hippocampal subfield. I used a protocol of stimulation which is widely known for inducing late-phase LTP in order to analyze whether FR had an impact on those forms of synaptic plasticity which requires protein sysntesis. As expected, FR led to significant increase in LTP expression also in the hippocampus (n FR=6 slices, 5 animals; n controls=4 slices, 3 animals; Two way ANOVA FR vs. controls $p<0.001$; Fig.8b) and the difference between the two groups was present since the few minutes after high frequency stimulation until the end of the recording, two hours later.

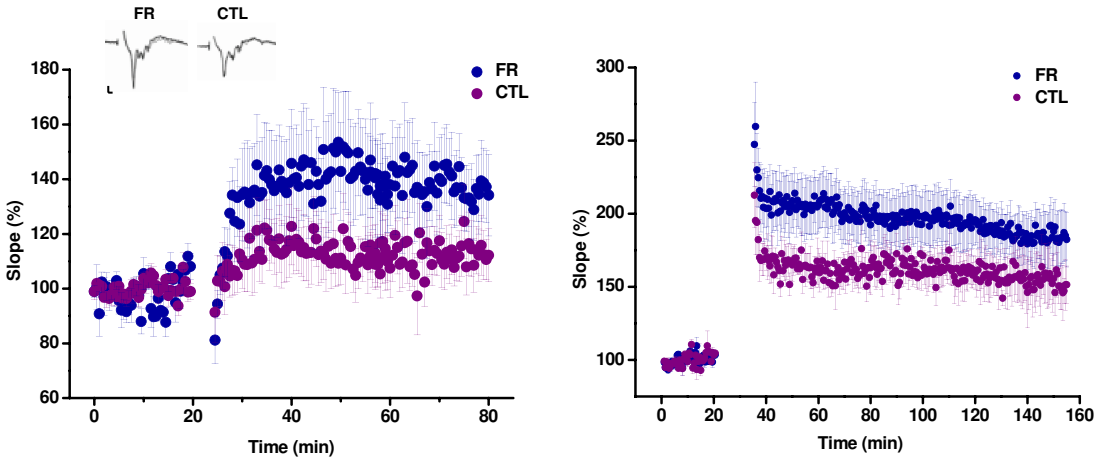

Figure 8: **FR is accompanied by increased LTP in the visual cortex and hippocampus**. A) Average time course of layer II-III field potential (FP) amplitude before and after Theta Burst Stimulation (TBS) of WM. Slices from FR rats showed potentiation of the response after TBS (slices=7; animals=5; Two way RM ANOVA, baseline vs. the last 20 min posttheta, p=0.025) differently from controls (slices=7, animals=5; Two way RM ANOVA, baseline vs. the last 20 min posttheta, p=0.218). The difference between posttheta response in FR animals and controls was statistically significant (Two way ANOVA in the last 20 min posttheta, p<0.001). The averaged FR amplitude after TBS were normalized to the mean value of the baseline recorded by each group. Sample traces before (thin line) and after (thick line) are also shown. Calibration bars: 200μV, 2.5msec. B) LTP in the CA1 subfield of the hippocampus was induced through stimulation of Schaffer collaterals and time course of field potential slope was recorded before and after High Frequency Stimulation (HFS). Slices from FR rats showed higher levels of potentiation after HFS (n=6 slices, 5 animals; Two way RM ANOVA, baseline vs. the last 20 min of posttheta, p=0.004) than controls (n=4 slices, 3 animals; Two way RM ANOVA, baseline vs. the last 20 min of posttheta, p=0.049). The difference between LTP in FR animals and controls was statistically significant (Two way ANOVA in the last 20 min of posttheta, p<0.001).

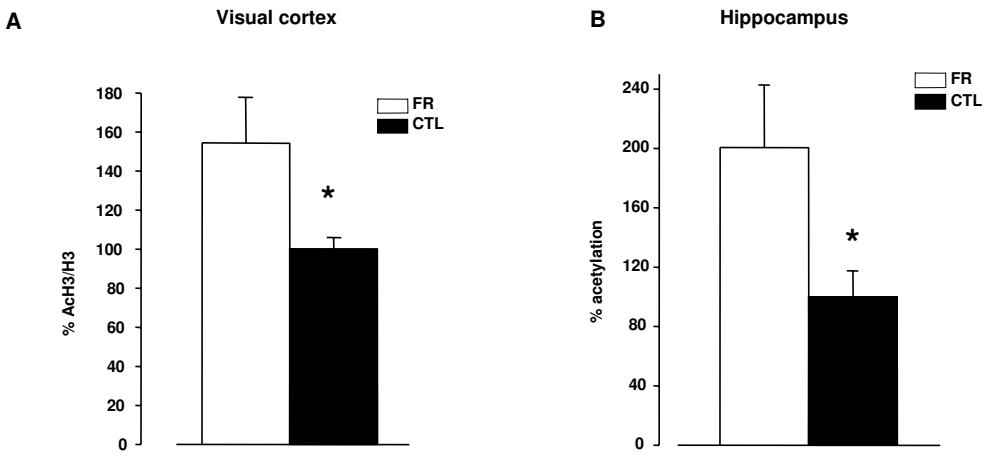

Figure 9: **FR induces histone acetylation in the visual cortex and hippocampus of adult rats**. A) Histone acetylation in the visual cortex was significantly increased in FR rats (n=6) with respect to controls (n=6; t-test p=0.048). Acetylation of FR was normalized to the mean value of controls. B) The hippocampus of FR rats showed significanltly higher levels of histone acetylation as compared to control animals (FR animals n=8; CTL animals n=8; t-test p=0.046). Error bars represent SEM; asterisks indicate statistical significance.

FR enhances histone acetylation in the visual cortex and hippocampus of adult rats

Histone posttranslational modifications regulate chromatin susceptibility to transcription with high levels of histone acetylation on a specific DNA segment being generally correlated with increased transcription rates (Mellor 2006; Workman 2006). Visual experience activates histone acetylation in the visual cortex during the critical period, but this capacity is downregulated in adult animals (Putignano et al. 2007). Moreover trichostatin treatment, which promotes histone acetylation, also enhances plasticity in the adult visual cortex (Putignano et al. 2007).

To analyze the effect of FR on histone acetylation we have measured the levels of acetylation on the Lys9 of the histone H3 in the visual cortex and the hipocampus of adult rats subjected to FR. Samples taken from adult animals under normal dietetic regimen were used as controls. As shown in the fig.9, the FR regimen determined a statistically significant increase in histone acetylation in both area tested, confirming that the action of FR is not limited to the visual system both at the functional an cellular level.

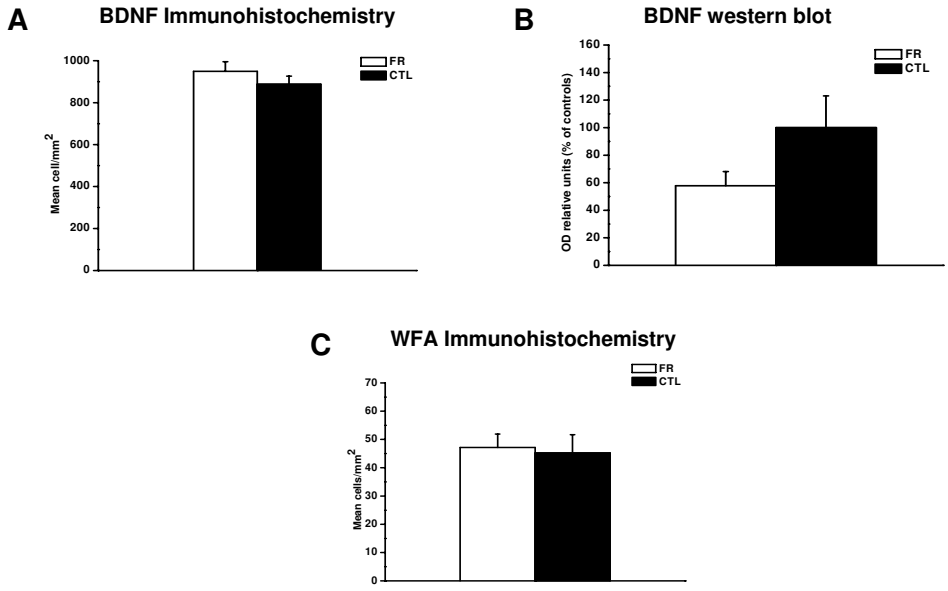

Figure 10: **Levels of BDNF protein and WFA in the visual cortex of adult FR rats and controls.**. A) Quantitative immunohistochemistry for BDNF revealed no difference between FR rats (cell density 949±46 cell/mm^2; n=6) and controls (cell density 888±38 cell/mm^2; n=6; t-test p=0.330) B) Analysis of BDNF protein by means of western blot showed no significant difference between FR animals (n=4) and control rats (n=4; t-test p=0.145). C) The density of WFA labeled cells was not different between the two groups as assessed by means of quantitative immunohistochemistry (cell density in FR rats 47±5 cell/mm^2, n=6; cell density in controls 45±6 cell/mm^2, n=6; t-test p=0.812). Error bars represent SEM.

Effects of FR on extracellular matrix remodeling and neurotrophin expression

Restoration of plasticity in the adult has been associated with an increase in the level of neurotrophins (Sale et al., 2007a; Maya-Vetencourt et al. 2008) and with remodeling of the extracellular matrix (Pizzorusso et al., 2002; Sale et al., 2007a).

I tested whether FR affected BDNF expression in the adult visual cortex by means of immunohistochemistry and western blot. No difference in BDNF was detectable between the two groups using both methods (Fig.10a,b).

Similarly, immunohistochemistry for WFA did not show any difference between FR rats and controls (Fig.10c). Thus, we conclude that our short term protocol of FR was unable to induce changes in BDNF expression or PNNs composition.

DISCUSSION

Since the first work demonstrating that FR increased lifespan in mice (McCay et al., 1935), many mechanisms have been proposed as the biological basis of FR action. Although there is disagreement regarding the specific event underlying the life-extending actions of FR, most gerontologist agree to the following general basis: 1) FR extends life by slowing the rate of aging; 2) Aging results from progressive organismic molecular damage due to an imbalance between damaging and repair processes; 3) FR slows aging by decreasing damaging processes and/or by increasing protective and repair processes.

In their 1935 report, McCay et al. proposed that FR extends the life of rats by retarding their growth. This view was held by most gerontologists until the 1980s when it was shown that FR also extends the life of adult mice (Weindruch and Walford, 1982) and rats (Masoro et al., 1985).

In 1960 Berg and Simms proposed that FR exerted its effects by decreasing body fat content. Although they did not measure the body fat of their experimental animals, they based their hypothesis on the reasonable assumption that FR reduces its content and the growing evidence in humans that a high level of fat has a negative impact on health. It was subsequently shown that FR does decrease body fat content of rats and mice (Bertrand et al., 1980; Harrison et al., 1984: Garthwaite et al., 1986) and that it is particularly effective in decreasing visceral fat (Barzilai and Gupta, 1999). FR has similar effects on body fat of rhesus and cynomolgus monkeys (Hansen and Bodkin, 1993; Verdery et al., 1997; Cefalu et al., 1997; Colman et al., 1999).

Many nutritionists viewed this hypothesis very favorably until two studies, published in the 1980s provided a strong case against its validity. Bertrand et al., (1980) reported no correlation between the body fat mass and the length of life of ad libitum

fed male F344 rats and a positive correlation in male rats of this strain maintained on FR regimen. Harrison et al. (1984) compared obese *ob/ob* mice with lean mice that were congenic except for the (*ob/ob*) locus. The length of life of the ad libitum fed *ob/ob* mice was less than that of the ad libitum fed lean mice, but obese mice on FR lived longer than the ad libitum fed lean mice, even though they had a greater body fat content. Indeed, the *ob/ob* mice on FR lived at least as long as FR lean mice.

These findings led to a dismissal of the *Reduction of Body Fat Hypothesis* until recently when the adipose tissue has been recognized as one of the most important source of those peripheal hormones which can influence feeding behaviour and metabolism. Thus, in my opinion, the reduction of body fat content observed in FR, since it is followed by hormonal modulation, needs a more accurate study in order to identify the realtive importance of paracrine signaling both for the extension of life and for plasticity in the CNS.

In 1979, Sacher and Duffy proposed that FR extends life by decreasing the metabolic rate. Several studies revealed that calorie restriction was associated with energy conservation (Gonzales-Pacheco et al., 1993; Santos-Pinto et al., 2001) and that mitochondria isolated from calorie-restricted animals produced less ATP than those from controls fed ad libitum (Sreekumar et al., 2002; Drew et al., 2003). However, separate investigations in rodents have suggested that, when adjusted for body weight, metabolic rate does not decrease with calorie restriction (Masoro et al., 1982; McCarter et al., 1985; Masoro, 1993). More importantly, calorie restriction prevents the age related decline in oxidative metabolism in muscle (Hepple et al., 2005; Baker et al., 2006). These data are supported by recent studies indicating that, in contrast to isolated mitochondria, ATP synthesis in intact myocytes and in vivo does not decrease following calorie restriction (Lopez-Lluch et al., 2006; Zangarelli et al., 2006). Additional support is provided by the finding that, in yeast, oxidative metabolism increases with calorie restriction (Lin et al., 2002a).

Altough FR does not seem to influence the cellular metabolic rate, it has been demonstrated that it has a beneficial effect on oxidative stress. In 1956, indeed, Harman proposed that aging occurs because of the accumulation in living organisms of oxidative damage caused by the free radicals that are continuously generated during the course

of metabolism. Over the years this theory of aging has gained favor and is currently held by many biogerontologist. Thus, it is not surprising that investigators studying FR have proposed that improved mitochondrial function, leading to decreased production of reactive oxygen species (ROS) and increased energy output is the basis of the life-extension triggered by FR. The superoxide anion radical is normally produced in low concentrations during oxidative phosphorylation, but levels increase substantially following mitochondrial damage, for example following intracellular calcium overload caused by excitotoxic injury (Balaban et al., 2005; Nicholls, 2004). Superoxide is subsequently converted to hydrogen peroxide, a source of hydroxyl radicals. The resultant oxidative damage to proteins, lipids and DNA leads to manifestations of neurological disease (Keller et al., 2005; Mariani et al., 2005; Reddy, 2006). A significant proportion of the neurological deficits that occur following stroke, head trauma, anoxia or even in Alzheimer's disease can in fact be attributed to secondary injury caused by glutamate excitotoxicity and, consequently, intracellular calcium overload, mitochondrial dysfunction and oxidative stress (Calabrese et al., 2001; Bramlett and Dietrich, 2004; Canevari et al., 2004).

Calorie restriction delays age-related oxidative damage to DNA, proteins and lipids, as evidenced by decreased tissue concentrations of peroxidized lipids, protein carbonyls and damaged bases in nuclear and mitochondrial DNA (Merry, 2004; Hunt et al., 2006). Several mechanisms have been proposed to explain antioxidant properties of calorie restriction. First, some studies suggested that calorie restriction enhances antioxidant defenses, including superoxide dismutase, glutathione peroxidase and catalase (Gong et al., 1997; Sreekumar et al., 2002; Agarwal et al., 2005; Rankin et al., 2006), although others found no significant effects (Sohal et al., 1994; Deruisseau et al., 2006). Second, a decrease in the mitochondrial production of ROS has been demonstrated, specifically at complex I of the respiratory chain (Sohal et al., 1994; Merry, 2002; Lambert and Merry, 2004; Gredilla and Barja, 2005). Brain mitochondria isolated from aged, calorie restricted rats produced significantly less hydrogen peroxide than those from controls fed ad libitum in the presence of pyruvate and malate, but not in the presence of succinate, consistent with an effect of calorie restriction at complex I (Sanz et al., 2005). The same conclusion was reached in studies of liver and heart

mitochondria (Gredilla et al., 2001; Lopez-Torres et al., 2002).

How calorie restriction actually decreases mitochondrial production of ROS is unclear, but the mechanism may involve uncoupling proteins (UCPs) which span the mitochondrial inner membrane and allow the leakage of protons from the intermembrane space to the matrix, thereby dissociating the electrochemical gradient (proton motive force) from ATP generation. This uncoupling diminishes the mitochondrial membrane potential and decreases the production of ROS (Harper et al., 2004; Andrews et al., 2005; Bevilacqua et al., 2005; Krauss et al., 2005; Lopez-Lluch et al., 2006). Consistently, enhanced UCP activity has been associated with increased longevity and neuronal resistance to ischemic, toxic, traumatic and epileptic injury (Mattiasson et al., 2003; Sullivan et al., 2003; Andrews et al., 2006; Conti et al., 2005, 2006; Liu et al., 2006). Further, mild mitochondrial uncoupling using the protonophore 2,4-dinitrophenol decreases ROS levels and enhances longevity (Caldeira da Silva et al., 2008). Although the effects of FR on ATP generation might appear to contradict those invoking uncoupling proteins, this discrepancy can be explained by the fact that calorie restriction also promotes mitochondrial biogenesis, thereby maintaining total metabolic output per cell while decreasing mitochondrial production of ROS (Diano et al., 2003; Nisoli et al., 2005; Civitarese et al., 2007; Valle et al., 2008).

Interestingly, the influence of FR on mithocondrial metabolism may also influence brain plasticity. The outgrowth of axons and the active transport of various molecules from the cell body to the synapse and back requires energy. These processes include the polymerization of actin filaments and microtubules, interactions of actin and myosin, and the operation of motor proteins such as kinesin and dynein, which propel cargo along cytoskeletal tracks (Pfister, 1999; Pollard et al., 2000; Pantaloni et al., 2001; Farrell et al., 2002). In a reciprocal manner, cytoskeletal proteins play a major role in controlling the location of mitochondria (Hollenbeck, 1996). Mitochondria are actively transported in axons in both anterograde and retrograde directions. Importantly, the size and location of mitochondria within presynaptic terminals change in ways that suggest specific adaptations of mitochondria for specific localized functions (Brodin et al., 1999; Nguyen et al., 1997). Synaptic localization and activity of mitochondria are essential for synaptic activity and dendritic spine remodeling, and it

seems that, reciprocally, synaptic activity modulates motility and fission/fusion balance in the mitochondria (Li et al., 2004). Furthermore, mitochondrial activity is important during the learning processes (Fride et al., 1989), and its inhibition impairs cognitive performance (Bennett and Rose 1992; Weeber et al., 2002; Levy et al., 2003). Recently, using greenfluorescence-labeled mitochondria and confocal technology, Tong (2007) has demonstrated that tetanic stimulation triggers a fast delivery of mitochondria to the synapse. The importance of mitochondrial delivery and activity is demonstrated by the suppression of both mitochondrial transport and potentiation of the synapse by rotenone. The use of mutant mice for porins, also called voltage-dependent anion channels, and cyclosporin A has demonstrated that the normal mitochondrial permeability transition pore complex function is required for synaptic plasticity and learning and memory processes (Weeber et al., 2002; Levy et al., 2003).

In spite of the many hypothesis proposed and investigated, the mechanism underlying the life-prolonging and anti-aging actions of FR remains unknown. It is likely that most of the described theories identify processes that play some role in the phenomenon which involve a set of more complex events. The capability to survive food deprivation, indeed, is considered a primitive advantageous adaptation (Holliday et al., 2006), a component of the homeodynamic response of living systems to environmental changes (Rattan et al., 2008). Almost all organisms, have the intrinsic faculty to respond, counteract and/or adapt to external and internal sources of disturbance usually referred to as stress. While a successful response to low doses of stressors improve the overall homeodynamics of individuals, an incomplete or failed response due to high levels or chronic stress leads to damaging and harmful effects. This bifasic response is very similar to that observed for a wide variety of physical, chemical and biological components (Calabrese and Baldwin, 2001a, b; Calabrese and Blain, 2005; Calabrese, 2004, 2005; Calabrese et al., 2006) and it has been defined with the term hormesis. In other words, hormesis refers to the phenomen whereby a usually detrimental environmental agent (radiation, chemical substance, food deprivation, etc.) changes its role to provide beneficial effects when administered at low intensities or concentrations (Furst, 1987). Thus, it has been hypothesized that if biological systems are deliberately exposed to mild stress, so that their homeodynamic pathways of maintenance and repair

are challenged and activated, a beneficial effect is triggered. Strong support to the idea that FR can be considered a low intensity stressor comes from findings in both rats and mice that FR causes the daily elevation of circadian peak plasma free corticostrone levels (Sabatino et al., 1991; Han et al., 1995; Patel and Finch 2002; Stamp et al., 2008). Furthermore, it has been demonstrated that rodents on FR regimen have an enhanced capability to cope with intense stressors (Masoro, 1998; Heydari et al., 1993; Klebanov et al., 1995). In the present work I show that, following an intermmitent fasting regimen, the increase in blood corticosterone is not continuous during time being present only at the end of a fasting day and returning to normality after one day of feeding thus confirming that FR is not a source of chronic stress. Moreover, the elevation of corticosterone levels does not result in the typical stress behavioural response since the performace in the elevated plus maze task (a validated method to measure anxiety-like behaviours in rodents, Walf and Frye, 2007) is similar in FR rats and controls.

Also the recovery of learning and memory in aged animals is part of the complex effects triggered by FR. This phenomenon may be due either to the slowing of senescence or to the increase in the overall plastic potential. With regard to this, it is interesting to understand if the actions of FR on brain plasticity are also present in an adult, not aged organism. This kind of analysis may have a double purpose: on one hand it could help in elucidating the real influence of FR on CNS function, on the other hand it is useful for a deeper understanding of the aging process itself.

In this study, I have shown that the positive outcome of FR is not limited to senescence since, in my model, a reduction of caloric intake is associated with increased plasticity in adult, not aged, healthy animals. I have demonstrated that a short-term protocol of FR started in adult age can restore plasticity in the visual system both at the level of synapses (through the renewed capability in inducing WM-LTP in the visual cortex) and at the level of function, since the system regains its juvenile responsiveness to MD. Interestingly, the increase in plasticity has been proven to be effective in the treatment of amblyopia since FR amblyopic rats showed a complete recovery of visual functions (binocularity and visual acuity) after reverse suture.

The effects of FR were not limited to the visual system being the enhancement

of synaptic plasticity also present in the hippocampus. This suggests that the dietary regimen could have a general impact on brain function promoting experience-dependent modifications of neuronal circuits also in adulthood.

As already said, the mechanisms by which dietary restriction leads to both an increased maximal and average lifespan with suppression of age-related pathologies and restoration of plasticity to the adult remain unclear. If from one hand it is unlikely that the beneficial effects of fasting operate via a single pathway, the research for mediators can be restricted to some good candidates. It is worth noting that, in this study, FR has been associated with a striking elevation in the rate of histone acetylation both in the visual cortex and in the hippocampus giving a general explanation of its wide range of effects. In line with our results, moreover, it has been demonstrated that the use of histone deacetylase inhibitors causes not only a lifespan extension (Zhao et al., 2005), but also an increase in adult brain plasticity (Putignano et al., 2007).

The results presented, are quite similar to those obtained with other protocols, including environmental enrichment (EE), both at the level of functional reactivation of plasticity in adulthood and at the level of increase in histone acetylation. Indeed, it has been demonstrated that EE in adult amblyopic rats restored normal visual acuity and ocular dominance (Sale et al., 2007a) and it has been correlated with chromatin modifications (histone-tail acetylation) (Fischer et al., 2007).

This similarities are not due to increased physical activity. Although FR has been associated with food anticipatory hyperactivity (Mistlberger, 1994; Challet et al., 1997; de Groot and Rusak, 2004;), I have demonstrated that the total mean daily locomotor activity in FR rats is even lower than in controls. Moreover, a very recent study demonstrate that FR-induced hyperactivity is critically and quantitatively dependent on corticosterone (Duclos et al., 2009) which undergoes dramatic oscillations in my model.

Thus, it seems that FR and EE, altough very different in their impact on the animal behavior, share some common mechanisms linked to the plastic outcome. In the last few years, the elevation in excitatory/inhibitory balance in the brain, has been increasingly recognized as crucial in the restoration of plasticity to the adult (He et al., 2006, 2007; Sale et al., 2007a; Maya-Vetencourt et al., 2008) and in the improve-

ment of neurodegenerative disorders (Enhinger et al., 2008). It has been proposed that the inhibitory tone surpasses two functional treshold during the development of the central nervous system. In early phases a proper level of inhibition is necessary to detect changes in neuronal activity, while the further maturation of GABAergic circuitry closes the critical period by reducing overall activity levels (Hensch et al., 1998; Hanover et al., 1999; Huang et al., 1999; Fagiolini and Hensch, 2000; Rozas et al., 2001). Notheworthy, it has been recently demonstarted that the inhibitory tone keeps its non permissive action with respect to plasticity also during adulthood. The pharmacological reduction of inhibition in the visual cortex of adult rats is, indeed, sufficient to restore ocular dominace shift in response to MD (Harauzov et al., in press).

Dark rearing during adult age, EE and fluoxetine administration have been proven to induce plasticity in the adult visual cortex and, notably, they have all been associated with a reduction of the inhibitory tone. In this work, I show that also FR is accompanied with a decrease in the cortical release of GABA suggesting that this event is crucial and might be even necessary for the plastic outcome. More interestingly, some of the molecular mechanisms associated with recovery of plasticity in EE and FR are quite different. FR animals, in fact, did not show any change either in BDNF or WFA labelling.

The result on WFA is in agreement with the work by Harauzov and collegues (in press) in which authors demonstrate that 3 days of MD accompanied by a decrease in GABAergic tone are sufficient in shifting the OD distribution but not in causing a remodeling of the extracellular matrix suggesting that this phenomenon may be just a consequence of the plastic event and is not necessary for its occurrence. Conversely, the lack of changes in BDNF expression is very surprising because it has been demonstrated that the the infusion of BDNF in the visual cortex of adult rats can lead per se to the induction of plasticity (Maya-Vetencourt et al., 2008). In spite of this striking result, the implication of neurotrophins in brain plasticity is still a controversial argument. It has been demonstrated, for example, that trkB signaling is required for amblyopia recovery but not for loss of cortical responses after MD (Kaneko et al., 2008). Moreover, the relationship between BDNF and decrease in inhibition has still to be clarified since BDNF overexpressing mice display an acceleration of critical period closure with

precocious maturation of inhibitory interneurons (Huang et al, 1999). One possible explanation of this contrasting results is that BDNF could have different roles, with respect to plasticity, during development and adulthood and this could be due to the dramatic changes that occur in the anatomical and functional properties of a developing brain. Furthermore, the fact that BDNF seems to be not always necessary to trigger functional plasticity, does not exclude that, in some cases, it could be sufficient to that.

As regard to FR, the lack of enhancement in BDNF expression observed in my model could be due to the particular short protocol of dietetic regimen used. Indeed, all works showing an increase in BDNF signaling after FR, used either aged mice maintained under dietary regimen for their whole life or adult animals restricted for 3 months (Lee et al., 2000; Duan et al., 2001a, b; Lee et al., 2002). The only study in which shorter periods of FR were used demonstrated an enhancement of BDNF signaling after spinal chord injury (Plunet et al., 2008). Thus, it is possible that BDNF could be one crucial molecular mediator of FR neuroprotective activity during ageing or injury-releated neurodegeneration, while it might be not required for the effects on plasticity.

The literature and the results described in this thesis show that FR increase longevity in mammals, slows their aging and enable them to avoid age-related diseases. Furthermore, I have demonstrated that it increases brain plasticity during adulthood. Studies should soon confirm the increased longevity and retardation of aging in primates and there is no reason to think the situation will be different in humans. Experiments of undernutrition without malnutrition in humans are rare, and although most of these studies do not survive critical examination (Roth et al., 1999), there is at least one that appear to be valid: Biosphere-2, a hermetically sealed compound located in Arizona that was intended to reproduce a complex ecosystem (tropical forest, desert, etc...), in which four men and four women lived for a two-year period. All their food was produced inside the compound. Because of some difficulties, their calorie ration fell to 1800 kcal per day at the beginning of the study and reached only 2200 kcal at the end of the experiment. Complete medical examinations were conducted regularly by one of the subjects who was a physician. During their stay in Biosphere-2,

subjects weight, body temperature, blood pressure, blood glucose, insulin, cholesterol and lipoprotein levels were diminished; these results are similar to those obtained in rodents and to some of those obtained in macaques (Walford et al., 2002). After the subjects left Biosphere-2, these variables gradually returned to their previous values. The authors of the Biosphere-2 study concluded that severe calorie restriction does not negatively affect health as long as other aspects of nutrition remain adequate; they also noted that the subjects performed substained physical activity even under these dietary conditions. These result, of course, provide no information about aging or plasticity, except for the fact that one of the perticipants was 67 years of age at the beginning of the study and experienced no particular problems.

The persons involved in this study were certainly highly motivated, or they would have left Biosphere-2 once the draconian nature of the regimen became clear. Moreover, the idea of subjecting the whole humanity to a calorie-restricted diet is unrealistic and it could be even very riskful if the regimen is not followed by a physician. Nevertheless, I have demonstrated that even a short period of FR could extert striking effects on brain physiology and this information is very crucial for the designation of FR as a therapeuthic tool. My results suggest a potential clinical application for FR in rehabilitation from central nervous system injuries being in particular useful as additional treatment to the motor therapy in all those cases in which the motor functions are partially or totally compromised. This observation is confirmed by studies in animal models demonstrating that FR following spinal cord injury could promote the recovery of motor functions (Plunet et al., 2008) and that maintenance of adult animals on a FR regimen resulted in reduced brain damage and improved behavioral outcome in a model of ischemic stroke (Yu and Mattson, 1999).

BIBLIOGRAPHY

Agarwal S, Sharma S, Agrawal V, Roy N. (2005). Caloric restriction augments ROS defense in S. cerevisiae, by a Sir2p independent mechanism. *Free Radic Res* 39, 55 – 62.

Ahmed AK, Guison NG, Yamadori T. (1996). A retrograde fluorescent-labeling study of direct relationship between the limbic (anterodorsal and anteroventral thalamic nuclei) and the visual system in the albino rat. *Brain Res* 729, 119 – 23.

Allison DB, Miller RA, Austad SN, Bouchard C, Leibel R, Klebanov S, Johnson T, Harrison DE. (2001). Genetic variability in responses to caloric restriction in animals and in regulation of metabolism and obesity in humans. *J Gerontol A Biol Sci Med Sci* 56, 55 – 65.

Al-Regaiey KA, Masternak MM, Bonkowski M, Sun L, Bartke A. (2005). Long-lived growth hormone receptor knockout mice: interaction of reduced insulin-like growth factor i/insulin signaling and caloric restriction. *Endocrinology* 146, 851 – 60.

Alvarez-Buylla A, Kirn JR. (1997). Birth, migration, incorporation, and death of vocal control neurons in adult songbirds. *J Neurobiol* 33, 585 – 601.

Anderson SP, Yoon L, Richard EB, Dunn CS, Cattley RC, Corton JC. (2002). Delayed liver regeneration in peroxisome proliferator-activated receptor-alpha-null mice. *Hepatology* 36, 544 – 54.

Andrews ZB, Horvath B, Barnstable CJ, Elsworth J, Yang L, Beal MF, Roth RH, Matthews RT, Horvath TL. (2005). Uncoupling protein-2 is critical for nigral dopamine cell survival in a mouse model of Parkinson's disease. *J Neurosci* 25, 184 – 91.

Andrews ZB, Rivera A, Elsworth JD, Roth RH, Agnati L, Gago B, Abizaid A, Schwartz M, Fuxe K, Horvath TL. (2006). Uncoupling protein-2 promotes nigrostriatal dopamine neuronal function. *Eur J Neurosci* 24, 32 – 6.

Anson RM, Guo Z, de Cabo R, Iyun T, Rios M, Hagepanos A, Ingram DK, Lane MA, Mattson MP. (2003). Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proc Natl Acad Sci U S A* 100, 6216 – 20.

Antonini, A., Fagiolini, M. and Stryker, MP. (1999). Anatomical correlates of functional plasticity in mouse visual cortex. *J Neurosci* 19, 4388 – 406.

Argentino DP, Dominici FP, Al-Regaiey K, Bonkowski MS, Bartke A, Turyn D. (2005). Effects of long-term caloric restriction on early steps of the insulin-signaling system in mouse skeletal muscle. *J Gerontol A Biol Sci Med Sci* 60, 28 – 34.

Artola A, Singer W. (1987). Long-term potentiation and NMDA receptors in rat visual cortex. *Nature* 330, 649 – 52.

Baker DJ, Betik AC, Krause DJ, Hepple RT. (2006). No decline in skeletal muscle oxidative capacity with aging in long-term calorically restricted rats: effects are independent of mitochondrial DNA integrity. *J Gerontol A Biol Sci Med Sci* 61, 675 – 84.

Balaban RS, Nemoto S, Finkel T. (2005). Mitochondria, oxidants, and aging. *Cell* 120, 483 – 95.

Barth M, Hirsch HV, Meinertzhagen IA, Heisenberg M. (1997). Experience-dependent developmental plasticity in the optic lobe of Drosophila melanogaster. *J Neurosci* 17, 1493 – 504.

Bartoletti A, Medini P, Berardi N, Maffei L. (2004). Environmental enrichment prevents effects of dark-rearing in the rat visual cortex. *Nat Neurosci* 7, 215 – 6.

Barzilai N, Gupta G. (1999). Revisiting the role of fat mass in the life extension induced by caloric restriction. *J Gerontol A Biol Sci Med Sci* 54, *B*89 – 96.

Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Tailor P, Morimoto RI, Cohen GM, Green DR. (2000). Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2, 469 – 75.

Benevento LA, Bakkum BW, Cohen RS. (1995). gamma-Aminobutyric acid and somatostatin immunoreactivity in the visual cortex of normal and dark-reared rats. *Brain Res* 689, 172 – 82.

Bennett MC, Rose GM. (1992). Chronic sodium azide treatment impairs learning of the Morris water maze task. *Behav Neural Biol* 58, 72 – 5.

Berardi, N., Pizzorusso, T. and Maffei, L. (2000). Critical periods during sensory development. *Curr Opin Neurobiol* 10, 138 – 45.

Berardi N, Pizzorusso T, Ratto GM, Maffei L. (2003). Molecular basis of plasticity in the visual cortex. *Trends Neurosci* 26, 368 – 78.

Berardi N, Braschi C, Capsoni S, Cattaneo A, Maffei L. (2007). Environmental enrichment delays the onset of memory deficits and reduces neuropathological

hallmarks in a mouse model of Alzheimer-like neurodegeneration. *J Alzheimers Dis* 11, 359 – 70.

Berg BN, Simms HS. (1960). Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of food intake. *J Nutr* 71, 255 – 63.

Berlucchi G, Buchtel HA. (2009). Neuronal plasticity: historical roots and evolution of meaning. *Exp Brain Res* 192, 307 – 19.

Bertrand HA, Lynd FT, Masoro EJ, Yu BP. (1980). Changes in adipose mass and cellularity through the adult life of rats fed ad libitum or a life-prolonging restricted diet. *J Gerontol* 35, 827 – 35.

Bevilacqua L, Ramsey JJ, Hagopian K, Weindruch R, Harper ME. (2005). Long-term caloric restriction increases UCP3 content but decreases proton leak and reactive oxygen species production in rat skeletal muscle mitochondria. *Am J Physiol Endocrinol Metab* 289, *E*429 – 38.

Björntorp P. (1991). Metabolic implications of body fat distribution. *Diabetes Care* 14, 1132 – 43.

Blüher M, Kahn BB, Kahn CR. (2003). Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299, 572 – 4.

Bokov A, Chaudhuri A, Richardson A. (2004). The role of oxidative damage and stress in aging. *Mech Ageing Dev* 125, 811 – 26.

Bordone L, Guarente L. (2005). Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat Rev Mol Cell Biol* 6, 298 – 305.

Bough KJ, Valiyil R, Han FT, Eagles DA. (1999). Seizure resistance is de-

pendent upon age and calorie restriction in rats fed a ketogenic diet. *Epilepsy Res* 35, 21 – 8.

Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB. (2002). Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* 416, 636 – 40.

Bramlett HM, Dietrich WD. (2004). Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. *J Cereb Blood Flow Metab* 24, 133 – 50.

Brask J, Kristensson K, Hill RH. (2004). Exposure to interferon-gamma during synaptogenesis increases inhibitory activity after a latent period in cultured rat hippocampal neurons. *Eur J Neurosci* 19, 3193 – 201.

Brochu M, Poehlman ET, Ades PA. (2000). Obesity, body fat distribution, and coronary artery disease. *J Cardiopulm Rehabil* 20, 96 – 108.

Brodin L, Bakeeva L, Shupliakov O. (1999). Presynaptic mitochondria and the temporal pattern of neurotransmitter release. *Philos Trans R Soc Lond B Biol Sci* 354, 365 – 72.

Bronner LL, Kanter DS, Manson JE. (1995). Primary prevention of stroke. *N Engl J Med* 333, 1392 – 400.

Bruce-Keller AJ, Umberger G, McFall R, Mattson MP. (1999). Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. *Ann Neurol* 45, 8 – 15.

Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. (2004). Stress-dependent reg-

ulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303, 2011 – 5.

Calabrese EJ, Baldwin LA. (2001a). Hormesis: a generalizable and unifying hypothesis. *Crit Rev Toxicol* 31, 353 – 424.

Calabrese EJ, Baldwin LA. (2001b). Hormesis: U-shaped dose responses and their centrality in toxicology. *Trends Pharmacol Sci* 22, 285 – 91.

Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, Clark JB. (2001). Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. *Neurochem Res* 39, 739 – 64.

Calabrese EJ. (2004). Hormesis: a revolution in toxicology, risk assessment and medicine. *EMBO Rep*, $SpecNo : S37 - 40$.

Calabrese EJ. (2005). Cancer biology and hormesis: human tumor cell lines commonly display hormetic (biphasic) dose responses. *Crit Rev Toxicol* 35, 463 – 582.

Calabrese EJ, Blain R. (2005). The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview. *Toxicol Appl Pharmacol* 202, 289 – 301.

Calabrese EJ, Staudenmayer JW, Stanek EJ. (2006). Drug development and hormesis: changing conceptual understanding of the dose response creates new challenges and opportunities for more effective drugs. *Curr Opin Drug Discov Devel* 9, 117 – 23.

Caldeira da Silva CC, Cerqueira FM, Barbosa LF, Medeiros MH, Kowaltowski AJ. (2008). Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. *Aging Cell* 7, 552 – 60.

Caleo, M., Lodovichi, C. and Maffei, L. (1999). Effects of nerve growth factor on visual cortical plasticity require afferent electrical activity. *Eur J Neurosci* 11, 2979 – 84.

Cancedda, L., Putignano, E., Sale, A., Viegi, A., Berardi, N. and Maffei, L. (2004). Acceleration of visual system development by environmental enrichment. *J Neurosci* 24, 4840 – 8.

Canevari L, Abramov AY, Duchen MR. (2004). Toxicity of amyloid beta peptide: tales of calcium, mitochondria, and oxidative stress. *Neurochem Res* 29, 637 – 50.

Castrén E, Zafra F, Thoenen H, Lindholm D. (1992). Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. *Proc Natl Acad Sci U S A* 89, 9444 – 8.

Castrén E. (2004). Neurotrophic effects of antidepressant drugs. *Curr Opin Pharmacol* 4, 58 – 64.

Castrén E. (2005). Is mood chemistry? *Nat Rev Neurosci* 6, 241 – 6.

Cefalu WT, Bell-Farrow AD, Wang ZQ, Sonntag WE, Fu MX, Baynes JW, Thorpe SR. (1995). Caloric restriction decreases age-dependent accumulation of the glycoxidation products, N epsilon-(carboxymethyl)lysine and pentosidine, in rat skin collagen. *J Gerontol A Biol Sci Med Sci* 50, *B*337 – 41.

Cefalu WT, Wagner JD, Wang ZQ, Bell-Farrow AD, Collins J, Haskell D, Bechtold R, Morgan T. (1997). A study of caloric restriction and cardiovascular aging in cynomolgus monkeys (Macaca fascicularis): a potential model for aging research. *J Gerontol A Biol Sci Med Sci* 52, *B*10 – 9.

Challet E, Pevet P, Malan A. (1997). Effect of prolonged fasting and subsequent

refeeding on free-running rhythms of temperature and locomotor activity in rats. *Behav Brain Res* 84, 275 – 84.

Chalon S, Vancassel S, Zimmer L, Guilloteau D, Durand G. (2001). Polyunsaturated fatty acids and cerebral function: focus on monoaminergic neurotransmission. *Lipids* 36, 937 – 44.

Chapman T, Partridge L. (1996). Female fitness in Drosophila melanogaster: an interaction between the effect of nutrition and of encounter rate with males. *Proc Biol Sci* 263, 755 – 9.

Cicerone CM. (1976). Cones survive rods in the light-damaged eye of the albino rat. *Science* 10, 1183 – 5.

Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, Smith SR, Ravussin E; CALERIE Pennington Team. (2007). Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. *PLoS Med* 4, *e*76.

Ciucci F, Putignano E, Baroncelli L, Landi S, Berardi N, Maffei L. (2007). Insulin-like growth factor 1 (IGF-1) mediates the effects of enriched environment (EE) on visual cortical development. *PLoS One* 2, *e*475.

Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leevers SJ, Partridge L. (2001). Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science* 292, 104 – 6.

Coffer P. (2003). OutFOXing the grim reaper: novel mechanisms regulating longevity by forkhead transcription factors. *Sci STKE* 2003, *PE*39.

Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz

KT, Gorospe M, de Cabo R, Sinclair DA. (2004). Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 305, 390 – 2.

Colman RJ, Ramsey JJ, Roecker EB, Havighurst T, Hudson JC, Kemnitz JW. (1997). Body fat distribution with long-term dietary restriction in adult male rhesus macaques. *J Gerontol A Biol Sci Med Sci* 54, *B*283 – 90.

Combs TP, Pajvani UB, Berg AH, Lin Y, Jelicks LA, Laplante M, Nawrocki AR, Rajala MW, Parlow AF, Cheeseboro L, Ding YY, Russell RG, Lindemann D, Hartley A, Baker GR, Obici S, Deshaies Y, Ludgate M, Rossetti L, Scherer PE. (2003).A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology* 145, 367 – 83.

Conti B, Sugama S, Lucero J, Winsky-Sommerer R, Wirz SA, Maher P, Andrews Z, Barr AM, Morale MC, Paneda C, Pemberton J, Gaidarova S, Behrens MM, Beal F, Sanna PP, Horvath T, Bartfai T. (2005). Uncoupling protein 2 protects dopaminergic neurons from acute 1,2,3,6-methyl-phenyl-tetrahydropyridine toxicity. *J Neurochem* 93, 493 – 501.

Conti B, Sanchez-Alavez M, Winsky-Sommerer R, Morale MC, Lucero J, Brownell S, Fabre V, Huitron-Resendiz S, Henriksen S, Zorrilla EP, de Lecea L, Bartfai T. (2006). Transgenic mice with a reduced core body temperature have an increased life span. *Science* 314, 825 – 8.

Corton JC, Apte U, Anderson SP, Limaye P, Yoon L, Latendresse J, Dunn C, Everitt JI, Voss KA, Swanson C, Kimbrough C, Wong JS, Gill SS, Chandraratna RA, Kwak MK, Kensler TW, Stulnig TM, Steffensen KR, Gustafsson JA, Mehendale HM. (2004).Mimetics of caloric restriction include agonists of lipid-activated nuclear receptors. *J Biol Chem* 279, 46204 – 12.

Culmsee C, Monnig J, Kemp BE, Mattson MP. (2001). AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. *J Mol Neurosci* 17, 45 – 58.

Daitoku H, Hatta M, Matsuzaki H, Aratani S, Ohshima T, Miyagishi M, Nakajima T, Fukamizu A. (2004). Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc Natl Acad Sci U S A* 101, 10042 – 7.

Deegan JF 2nd, Jacobs GH. (1993). On the identity of the cone types of the rat retina. *Exp Eye Res* 56, 375 – 7.

de Groot MH, Rusak B. (2004). Housing conditions influence the expression of food-anticipatory activity in mice. *Physiol Behav* 83, 447 – 57.

Deruisseau KC, Kavazis AN, Judge S, Murlasits Z, Deering MA, Quindry JC, Lee Y, Falk DJ, Leeuwenburgh C, Powers SK. (2006). Moderate caloric restriction increases diaphragmatic antioxidant enzyme mRNA, but not when combined with lifelong exercise. *Antioxid Redox Signal* 8, 539 – 47.

Diamond MC. (2001). Response of the brain to enrichment. *An Acad Bras Cienc* 73, 211 – 20.

Diano S, Matthews RT, Patrylo P, Yang L, Beal MF, Barnstable CJ, Horvath TL. (2003). Uncoupling protein 2 prevents neuronal death including that occurring during seizures: a mechanism for preconditioning. *Endocrinology* 144, 5014–21.

Diao LH, Bickford PC, Stevens JO, Cline EJ, Gerhardt GA. (1997). Caloric restriction enhances evoked DA overflow in striatum and nucleus accumbens of aged Fischer 344 rats. *Brain Res* 763, 276 – 80.

Dityatev A, Schachner M. (2003). Extracellular matrix molecules and synaptic plasticity. *Nat Rev Neurosci* 4, 456 − 68.

Domenici, L., Cellerino, A. and Maffei, L. (1993). Monocular deprivation effects in the rat visual cortex and lateral geniculate nucleus are prevented by nerve growth factor (NGF). II. Lateral geniculate nucleus. *Proc Biol Sci* 251, 25 − 31.

Dreher B, Thong IG, Shameem N, McCall MJ. (1985). Development of cortical afferents and cortico-tectal efferents of the mammalian (rat) primary visual cortex.*Aust N Z J Ophthalmol* 13, 251 − 61.

Drew B, Phaneuf S, Dirks A, Selman C, Gredilla R, Lezza A, Barja G, Leeuwenburgh C. (2003). Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *m J Physiol Regul Integr Comp Physiol* 284, *R*474 − 80.

Droste SK, de Groote L, Atkinson HC, Lightman SL, Reul JM, Linthorst AC. (2008). Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress. *Endocrinology* 149, 3244 − 53.

Duan W, Mattson MP. (1999). Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J Neurosci Res* 57, 195 − 206.

Duan W, Guo Z, Mattson MP. (2001a). Brain-derived neurotrophic factor mediates an excitoprotective effect of dietary restriction in mice. *J Neurochem* 76, 619−26.

Duan W, Lee J, Guo Z, Mattson MP. (2001b). Dietary restriction stimulates BDNF production in the brain and thereby protects neurons against excitotoxic injury. *J Mol Neurosci* 16, 1 − 12.

Duan W, Ladenheim B, Cutler RG, Kruman II, Cadet JL, Mattson MP. (2002). Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. *J Neurochem* 80, 101 – 10.

Duan W, Guo Z, Jiang H, Ware M, Li XJ, Mattson MP. (2003). Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice. *Proc Natl Acad Sci U S A* 100, 2911 – 6.

Dubey A, Forster MJ, Lal H, Sohal RS. (1996). Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. *Arch Biochem Biophys* 333, 189 – 97.

Duclos M, Gatti C, Bessière B, Mormède P. (2009). Tonic and phasic effects of corticosterone on food restriction-induced hyperactivity in rats. *Psychoneuroendocrinology* 34, 436 – 45.

Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S. (1996). Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature* 383, 710 – 3.

Eckles-Smith K, Clayton D, Bickford P, Browning MD. (2000). Caloric restriction prevents age-related deficits in LTP and in NMDA receptor expression. *Brain Res Mol Brain Res* 78, 154 – 62.

Ehninger D, Li W, Fox K, Stryker MP, Silva AJ. (2008). Reversing neurodevelopmental disorders in adults. *Neuron* 60, 950 – 60.

Escher P, Braissant O, Basu-Modak S, Michalik L, Wahli W, Desvergne B. (2001). Rat PPARs: quantitative analysis in adult rat tissues and regulation in

fasting and refeeding. *Endocrinology* 142, 4195 – 202.

Escorihuela RM, Fernández-Teruel A, Tobeña A, Vivas NM, Mármol F, Badia A, Dierssen M. (1995). Early environmental stimulation produces long-lasting changes on beta-adrenoceptor transduction system. *Neurobiol Learn Mem* 64, 49 – 57.

Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L. and Maffei, L. (1994). Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. *Vision Res* 34, 709 – 20.

Fagiolini, M. and Hensch, T. K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. *Nature* 404, 183 – 6.

Farrell CM, Mackey AT, Klumpp LM, Gilbert SP. (2002). The role of ATP hydrolysis for kinesin processivity. *J Biol Chem* 277, 17079 – 87.

Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A. (1993). Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *J Biol Chem* 268, 26055 – 8.

Feldman, D. E. (2000). Inhibition and plasticity. *Nat Neurosci* 3, 303 – 4.

Fenech M. (2001). The role of folic acid and Vitamin B12 in genomic stability of human cells. *Mutat Res* 475, 57 – 67.

Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC. (2007). Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nat Neurosci* 10, 411 – 3.

Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH. (2007). Recovery

of learning and memory is associated with chromatin remodelling. *Nature* 447, 178−82.

Frenkel MY, Bear MF. (2004). How monocular deprivation shifts ocular dominance in visual cortex of young mice. *Neuron* 44, 917 − 23.

Fride E, Ben-Or S, Allweis C. (1989). Mitochondrial protein synthesis may be involved in long-term memory formation. *Pharmacol Biochem Behav* 32, 873 − 8.

Frydman J. (2001). Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem* 70, 603 − 47.

Fukuda Y, Hsiao CF, Hara Y, Iwama K. (1981). Properties of ipsilateral retinogeniculate afferents in albino and hooded rats. *Neurosci Lett* 22, 173 − 8.

Fukuoka M, Daitoku H, Hatta M, Matsuzaki H, Umemura S, Fukamizu A. (2003). Negative regulation of forkhead transcription factor AFX (Foxo4) by CBP-induced acetylation. *Int J Mol Med* 12, 503 − 8.

Furst A. (1987). Hormetic effects in pharmacology: pharmacological inversions as prototypes for hormesis. *Health Phys* 52, 527 − 30.

Furukawa-Hibi Y, Yoshida-Araki K, Ohta T, Ikeda K, Motoyama N. (2002). FOXO forkhead transcription factors induce G(2)-M checkpoint in response to oxidative stress. *J Biol Chem* 277, 26729 − 32.

Furuyama T, Kitayama K, Yamashita H, Mori N. (2003). Forkhead transcription factor FOXO1 (FKHR)-dependent induction of PDK4 gene expression in skeletal muscle during energy deprivation. *Biochem J* 375, 365 − 71.

Gage FH. (2000). Mammalian neural stem cells. *Science* 287, 1433 − 8.

Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, et al. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature* 373, 523 – 7.

Garthwaite SM, Cheng H, Bryan JE, Craig BW, Holloszy JO. (1986). Ageing, exercise and food restriction: effects on body composition. *Mech Ageing Dev* 36, 187 – 96.

Gething MJ. (1999). Role and regulation of the ER chaperone BiP. *Semin Cell Dev Biol* 10, 465 – 72.

Gianfranceschi L, Siciliano R, Walls J, Morales B, Kirkwood A, Huang ZJ, Tonegawa S, Maffei L. (2003). Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. *Proc Natl Acad Sci U S A* 100, 12486 – 91.

Giannakou ME, Goss M, Jünger MA, Hafen E, Leevers SJ, Partridge L. (2004). Long-lived Drosophila with overexpressed dFOXO in adult fat body. *Science* 305, 361.

Gilbert DL, Pyzik PL, Freeman JM. (2000). The ketogenic diet: seizure control correlates better with serum beta-hydroxybutyrate than with urine ketones. *J Child Neurol* 15, 787 – 90.

Gong X, Shang F, Obin M, Palmer H, Scrofano MM, Jahngen-Hodge J, Smith DE, Taylor A. (1997). Antioxidant enzyme activities in lens, liver and kidney of calorie restricted Emory mice. *Mech Ageing Dev* 99, 181 – 92.

Gonzales-Pacheco DM, Buss WC, Koehler KM, Woodside WF, Alpert SS. (1993). Energy restriction reduces metabolic rate in adult male Fisher-344 rats. *J Nutr* 123, 90 – 7.

Goodman CS, Shatz CJ. (1993). Developmental mechanisms that generate precise patterns of neuronal connectivity. *Cell* 72, 77 – 98.

Grant WB (1999). Dietary links to Alzheimer's disease: 1999 update. *J Alzheimers Dis* 1, 197 – 201.

Gredilla R, Barja G. (2005). Minireview: the role of oxidative stress in relation to caloric restriction and longevity. *Endocrinology* 146, 3713 – 7.

Gredilla R, Sanz A, Lopez-Torres M, Barja G. (2001). Caloric restriction decreases mitochondrial free radical generation at complex I and lowers oxidative damage to mitochondrial DNA in the rat heart. *FASEB J* 15, 1589 – 91.

Greene AE, Todorova MT, McGowan R, Seyfried TN. (2001). Caloric restriction inhibits seizure susceptibility in epileptic EL mice by reducing blood glucose. *Epilepsia* 42, 1371 – 8.

Greenough WT, Volkmar FR. (1973). Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Exp Neurol* 40, 491 – 504.

Gremlich S, Nolan C, Roduit R, Burcelin R, Peyot ML, Delghingaro-Augusto V, Desvergne B, Michalik L, Prentki M, Wahli W. (2005). Pancreatic islet adaptation to fasting is dependent on peroxisome proliferator-activated receptor alpha transcriptional up-regulation of fatty acid oxidation. *Endocrinology* 146, 375 – 82.

Guarente L, Kenyon C. (2000). Genetic pathways that regulate ageing in model organisms. *Nature* 408, 255 – 62.

Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, Mattson MP. (1999). Increased vulnerability of hippocampal neurons to excitotoxic necrosis in

presenilin-1 mutant knock-in mice. *Nat Med* 5, 101 – 6.

Guo Z, Ersoz A, Butterfield DA, Mattson MP. (2000). Beneficial effects of dietary restriction on cerebral cortical synaptic terminals: preservation of glucose and glutamate transport and mitochondrial function after exposure to amyloid beta-peptide, iron, and 3-nitropropionic acid. *J Neurochem* 75, 314 – 20.

Hajszan T, MacLusky NJ, Leranth C. (2005). Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. *Eur J Neurosci* 21, 1299 – 303.

Hale PT. (1980). Conduction velocities of rat retinal ganglion cells with uncrossed axons. *Brain Res* 201, 442 – 5.

Han ES, Levin N, Bengani N, Roberts JL, Suh Y, Karelus K, Nelson JF. (1995). Hyperadrenocorticism and food restriction-induced life extension in the rat: evidence for divergent regulation of pituitary proopiomelanocortin RNA and adrenocorticotropic hormone biosynthesis. *J Gerontol A Biol Sci Med Sci* 50, *B*288 – 94.

Halliwell B. (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging* 18, 685 – 716.

Hanover JL, Huang ZJ, Tonegawa S, Stryker MP. (1999). Brain-derived neurotrophic factor overexpression induces precocious critical period in mouse visual cortex. *J Neurosci* 19, *RC*40.

Hansen BC, Bodkin NL. (1993). Primary prevention of diabetes mellitus by prevention of obesity in monkeys. *Diabetes* 42, 1809 – 14.

Harauzov A, Spolidoro M, Di Cristo G, De Pasquale R, Cancedda L, Pizzorusso T, Viegi A, Maffei L. (in press). Reducing intracortical inhibition in the

adult visual cortex promotes ocular dominance plasticity. *J Neurosci*.

Harman D. (1956). Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 11, 298 – 300.

Harper ME, Bevilacqua L, Hagopian K, Weindruch R, Ramsey JJ. (2004). Ageing, oxidative stress, and mitochondrial uncoupling. *Acta Physiol Scand* 182, 321 – 31.

Harrison DE, Archer JR, Astle CM. (1984). Effects of food restriction on aging: separation of food intake and adiposity. *Proc Natl Acad Sci U S A* 81, 1835 – 8.

Härtig W, Brauer K, Brückner G. (1992). Wisteria floribunda agglutinin-labelled nets surround parvalbumin-containing neurons. *Neuroreport* 3, 869 – 72.

Härtig, W., Derouiche, A., Welt, K., Brauer, K., Grosche, J., Mader, M., Reichenbach, A. and Brückner, G. (1999). Cortical neurons immunoreactive for the potassium channel Kv3.1b subunit are predominantly surrounded by perineuronal nets presumed as a buffering system for cations. *Brain Res* 842, 15 – 29.

Hayhow WR, Sefton A, Webb C (1962). Primary optic centers of the rat in relation to the terminal distribution of the crossed and uncrossed optic nerve fibers. *J Comp Neurol* 118, 295 – 321.

He HY, Hodos W, Quinlan EM. (2006). Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex. *J Neurosci* 26, 2951 – 5.

He HY, Ray B, Dennis K, Quinlan EM. (2007). Experience-dependent recovery of vision following chronic deprivation amblyopia. *Nat Neurosci* 10, 1134 – 6.

Henderson ST, Johnson TE. (2001). daf-16 integrates developmental and envi-

ronmental inputs to mediate aging in the nematode Caenorhabditis elegans. *Curr Biol* 11, 1975 – 80.

Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S. and Kash, S. F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science* 282, 1504 – 8.

Hensch, T. K. (2004). Critical period regulation. *Annu Rev Neurosci* 27, 549 – 79.

Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. *Nat Rev Neurosci* 6, 877 – 88.

Hepple RT, Baker DJ, Kaczor JJ, Krause DJ. (2005). Long-term caloric restriction abrogates the age-related decline in skeletal muscle aerobic function. *FASEB J* 19, 1320 – 2.

Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M. (2001). CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 413, 179−83.

Heydari AR, Wu B, Takahashi R, Strong R, Richardson A. (1993). Expression of heat shock protein 70 is altered by age and diet at the level of transcription. *Mol Cell Biol* 13, 2909 – 18.

Hockfield, S., Kalb, R. G., Zaremba, S. and Fryer, H. (1990). Expression of neural proteoglycans correlates with the acquisition of mature neuronal properties in the mammalian brain. *Cold Spring Harb Symp Quant Biol* 55, 505 – 14.

Holderbach R, Clark K, Moreau JL, Bischofberger J, Normann C. (2005). Enhanced long-term synaptic depression in an animal model of depression. *Biol Psychiatry* 62, 92 – 100.

Hollenbeck PJ. (1996). The pattern and mechanism of mitochondrial transport in axons. *Front Biosci* 1, *d*91 – 102.

Holliday R. (2006). Food, fertility and longevity. *Biogerontology* 7, 139 – 41.

Hori N, Hirotsu I, Davis PJ, Carpenter DO. (1992). Long-term potentiation is lost in aged rats but preserved by calorie restriction. *Neuroreport* 3, 1085 – 8.

Houthoofd K, Braeckman BP, Lenaerts I, Brys K, De Vreese A, Van Eygen S, Vanfleteren JR. (2002). No reduction of metabolic rate in food restricted Caenorhabditis elegans. *Exp Gerontol* 37, 1359 – 69.

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 274, 99 – 102.

Huang, Z. J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M. F., Maffei, L. and Tonegawa, S. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* 98, 739 – 55.

Hubel, D. H., Wiesel, T. N. (1959). Receptive fields of single neurones in the cat's striate cortex. *J Physiol* 148, 574 – 91.

Hubel, D. H., Wiesel, T. N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J Physiol* 160, 106 – 54.

Hubel, D. H., Wiesel, T. N. (1963). Shape and arrangement of columns in cat's striate cortex. *J Physiol* 165, 559 – 68.

Hubel, D. H., Wiesel, T. N. (1970). The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J Physiol* 206, 419 – 36.

Hunt ND, Hyun DH, Allard JS, Minor RK, Mattson MP, Ingram DK, de Cabo R. (2006). TBioenergetics of aging and calorie restriction. *Ageing Res Rev* 5, 125 – 43.

Hursting SD, Perkins SN, Phang JM, Barrett JC. (2001). Diet and cancer prevention studies in p53-deficient mice. *J Nutr* 131, 3092S – 4S.

Idrobo F, Nandy K, Mostofsky DI, Blatt L, Nandy L. (1987). Dietary restriction: effects on radial maze learning and lipofuscin pigment deposition in the hippocampus and frontal cortex. *Arch Gerontol Geriatr* 6, 355 – 62.

Imae M, Fu Z, Yoshida A, Noguchi T, Kato H. (2003). Nutritional and hormonal factors control the gene expression of FoxOs, the mammalian homologues of DAF-16. *J Mol Endocrinol* 30, 253 – 62.

Imai S, Armstrong CM, Kaeberlein M, Guarente L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403, 795 – 800.

Improta T, Salvatore AM, Di Luzio A, Romeo G, Coccia EM, Calissano P. (1988). IFN-gamma facilitates NGF-induced neuronal differentiation in PC12 cells. *Exp Cell Res* 179, 1 – 9.

Ingram DK, Weindruch R, Spangler EL, Freeman JR, Walford RL. (1987). Dietary restriction benefits learning and motor performance of aged mice. *J Gerontol* 42, 78 – 81.

Iwai Y, Fagiolini M, Obata K, Hensch TK. (2003). Rapid critical period in-

duction by tonic inhibition in visual cortex. *J Neurosci* 23, 6695 – 702.

Jeffrey G. (1984). Transneuronal effects of early eye removal on geniculo-cortical projection cells. *Dev Brain Res* 13, 257 – 263.

Jenner P, Olanow CW. (1998). Understanding cell death in Parkinson's disease. *Ann Neurol* 44, *S*72 – 84.

Kaeberlein M, McVey M, Guarente L. (1999). The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev* 13, 2570 – 80.

Kaestner KH, Knochel W, Martinez DE. (2000). Unified nomenclature for the winged helix/forkhead transcription factors. *Genes Dev* 14, 142 – 6.

Kamei Y, Ohizumi H, Fujitani Y, Nemoto T, Tanaka T, Takahashi N, Kawada T, Miyoshi M, Ezaki O, Kakizuka A. (2003). PPARgamma coactivator 1beta/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity. *Proc Natl Acad Sci U S A* 100, 12378 – 83.

Kaneko M, Hanover JL, England PM, Stryker MP. (2008). TrkB kinase is required for recovery, but not loss, of cortical responses following monocular deprivation. *Nat Neurosci* 11, 497 – 504.

Kashiwaya Y, Takeshima T, Mori N, Nakashima K, Clarke K, Veech RL. (2000). D-beta-hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. *Proc Natl Acad Sci U S A* 97, 5440 – 4.

Katz LC, Shatz CJ. (1996). Synaptic activity and the construction of cortical circuits. *Science* 274, 1133 – 8.

Kayo T, Allison DB, Weindruch R, Prolla TA. (2001). Influences of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. *Proc Natl Acad Sci U S A* 98, 5093 – 8.

Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. (2005). Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* 64, 1152 – 6.

Kemnitz JW, Roecker EB, Weindruch R, Elson DF, Baum ST, Bergman RN. (1994). Dietary restriction increases insulin sensitivity and lowers blood glucose in rhesus monkeys. *Am J Physiol* 266, *E*540 – 7.

Kempermann G, Kuhn HG, Gage FH. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386, 493 – 5.

Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. (1993). A C. elegans mutant that lives twice as long as wild type. *Nature* 366, 461 – 4.

Kim HJ, Kim KW, Yu BP, Chung HY. (2000). The effect of age on cyclooxygenase-2 gene expression: NF-kB activation and IkBα degradation. *Free Radic Biol Med* 28, 683 – 92.

Kim H, Haluzik M, Asghar Z, Yau D, Joseph JW, Fernandez AM, Reitman ML, Yakar S, Stannard B, Heron-Milhavet L, Wheeler MB, LeRoith D. (2003). Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. *Diabetes* 52, 1770 – 8.

Kirkwood, A. and Bear, M. F. (1994). Hebbian synapses in visual cortex. *J Neurosci* 14, 1634 – 45.

Kirkwood, A., Lee, H. K. and Bear, M. F. (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature* 375, 328 – 31.

Klebanov S, Diais S, Stavinoha WB, Suh Y, Nelson JF. (1995). Hyperadrenocorticism, attenuated inflammation, and the life-prolonging action of food restriction in mice. *J Gerontol A Biol Sci Med Sci* 50, *B*79 – 82.

Kleschevnikov AM, Belichenko PV, Villar AJ, Epstein CJ, Malenka RC, Mobley WC. (2004). Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. *J Neurosci* 24, 8153 – 60.

Koponen E, Rantamäki T, Voikar V, Saarelainen T, MacDonald E, Castrén E. (2005). Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. *Cell Mol Biol* 25, 973 – 80.

Koponen E, Rantamäki T, Voikar V, Saarelainen T, MacDonald E, Castrén E. (2005). Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. *Cell Mol Biol* 25, 973 – 80.

Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, Huang TT, Bos JL, Medema RH, Burgering BM. (2002). Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419, 316 – 21.

Krauss S, Zhang CY, Lowell BB. (2005). The mitochondrial uncoupling-protein homologues. *Nat Rev Mol Cell Biol* 6, 248 – 61.

Kressler D, Schreiber SN, Knutti D, Kralli A. (2002). The PGC-1-related protein PERC is a selective coactivator of estrogen receptor alpha. *J Biol Chem* 277, 13918 – 25.

Kroetz DL, Yook P, Costet P, Bianchi P, Pineau T. (1998). Peroxisome proliferator-activated receptor alpha controls the hepatic CYP4A induction adaptive response to starvation and diabetes. *J Biol Chem* 273, 31581 − 9.

Kruman II, Kumaravel TS, Lohani A, Pedersen WA, Cutler RG, Kruman Y, Haughey N, Lee J, Evans M, Mattson MP. (2002). Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. *J Neurosci* 22, 1752 − 62.

Lambert AJ, Merry BJ. (2004). Effect of caloric restriction on mitochondrial reactive oxygen species production and bioenergetics: reversal by insulin. *Am J Physiol Regul Integr Comp Physiol* 286, *R*71 − 9.

Landi S, Cenni MC, Maffei L, Berardi N. (2007). Environmental enrichment effects on development of retinal ganglion cell dendritic stratification require retinal BDNF. *PLoS One* 2, *e*346.

Landry J, Sutton A, Tafrov ST, Heller RC, Stebbins J, Pillus L, Sternglanz R. (2000). The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci U S A* 97, 5807 − 11.

Lane MA, Ball SS, Ingram DK, Cutler RG, Engel J, Read V, Roth GS. (1995). Environmental enrichment effects on development of retinal ganglion cell dendritic stratification require retinal BDNF. *PLoS One* 2, *e*346.

Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnics Z, Lee VM, Hersh LB, Sapolsky RM, Mirnics K, Sisodia SS. (2005). Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell* 120, 701−13.

Lee CK, Klopp RG, Weindruch R, Prolla TA. (1999). Gene expression pro-

file of aging and its retardation by caloric restriction. *Science* 285, 1390 – 3.

Lee CK, Weindruch R, Prolla TA. (2000a). Gene-expression profile of the ageing brain in mice. *Nat Genet* 25, 294 – 7.

Lee J, Duan W, Long JM, Ingram DK, Mattson MP. (2000b). Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats. *J Mol Neurosci* 15, 99 – 108.

Lee J, Duan W, Mattson MP. (2002a). Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J Neurochem* 82, 1367 – 75.

Lee J, Seroogy KB, Mattson MP. (2002b). Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. *J Neurochem* 80, 539 – 47.

Lee J, Kim SJ, Son TG, Chan SL, Mattson MP. (2006). Interferon-gamma is up-regulated in the hippocampus in response to intermittent fasting and protects hippocampal neurons against excitotoxicity. *J Neurosci Res* 83, 1552 – 7.

Leone TC, Weinheimer CJ, Kelly DP. (1999). A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. *Proc Natl Acad Sci U S A* 96, 7473 – 8.

Levi F. (1999). Cancer prevention: epidemiology and perspectives. *Eur J Cancer* 35, 1912 – 24.

Levy M, Faas GC, Saggau P, Craigen WJ, Sweatt JD. (2003). Mitochondrial

regulation of synaptic plasticity in the hippocampus. *J Biol Chem* 278, 17727 – 34.

Li Z, Okamoto K, Hayashi Y, Sheng M. (2004). The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 119, 873 – 87.

Liao DS, Krahe TE, Prusky GT, Medina AE, Ramoa AS. (2004). Recovery of cortical binocularity and orientation selectivity after the critical period for ocular dominance plasticity. *J Neurophysiol* 92, 2113 – 21.

Libina N, Berman JR, Kenyon C. (2003). Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. *Cell* 115, 489 – 502.

Liepert J. (2006). Motor cortex excitability in stroke before and after constraint-induced movement therapy. *Cogn Behav Neurol* 19, 41 – 7.

Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC, Fink GR, Guarente L. (2002a). Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. *Nature* 418, 344 – 8.

Lin SJ, Ford E, Haigis M, Liszt G, Guarente L. (2004a). Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev* 18, 12 – 6.

Lin J, Puigserver P, Donovan J, Tarr P, Spiegelman BM. (2002b). Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. *J Biol Chem* 277, 1645 – 8.

Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jäger S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman

GI, Lowell BB, Krainc D, Spiegelman BM. (2004b). Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell* 119, 121−35.

Ling C, Poulsen P, Carlsson E, Ridderstråle M, Almgren P, Wojtaszewski J, Beck-Nielsen H, Groop L, Vaag A. (2004). Multiple environmental and genetic factors influence skeletal muscle PGC-1alpha and PGC-1beta gene expression in twins. *J Clin Invest* 114, 1518 − 26.

Liu J, Solway K, Messing RO, Sharp FR. (1998). Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. *J Neurosci* 18, 7768 − 78.

Liu D, Chan SL, de Souza-Pinto NC, Slevin JR, Wersto RP, Zhan M, Mustafa K, de Cabo R, Mattson MP. (2006). Mitochondrial UCP4 mediates an adaptive shift in energy metabolism and increases the resistance of neurons to metabolic and oxidative stress. *Neuromolecular Med* 8, 389 − 414.

Logroscino G, Marder K, Cote L, Tang MX, Shea S, Mayeux R. (1996). Dietary lipids and antioxidants in Parkinson's disease: a population-based, case-control study. *Ann Neurol* 39, 89 − 94.

López-Lluch G, Hunt N, Jones B, Zhu M, Jamieson H, Hilmer S, Cascajo MV, Allard J, Ingram DK, Navas P, de Cabo R. (2006). Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc Natl Acad Sci U S A* 103, 1768 − 73.

López-Torres M, Gredilla R, Sanz A, Barja G. (2002). Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. *Free Radic Biol Med* 32, 882 − 9.

Lowenstein DH, Chan PH, Miles MF. (1991). The stress protein response in cultured neurons: characterization and evidence for a protective role in excitotoxicity.

Neuron 7, 1053 – 60.

Lund RD. (1965). Uncrossed Visual Pathways of Hooded and Albino Rats.*Science* 149, 1506 – 1507.

Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L, Gu W. (2001). Negative control of p53 by Sir2alpha promotes cell survival under stress. *Cell* 107, 137 – 48.

Mahoney AW, Hendricks DG, Bernhard N, Sisson DV. (1983). Fasting and ketogenic diet effects on audiogenic seizures susceptibility of magnesium deficient rats. *Pharmacol Biochem Behav* 18, 683 – 7.

Majewska A, Sur M. (2003). Motility of dendritic spines in visual cortex in vivo: changes during the critical period and effects of visual deprivation. *Proc Natl Acad Sci U S A* 100, 16024 – 9.

Major DE, Kesslak JP, Cotman CW, Finch CE, Day JR. (1997). Life-long dietary restriction attenuates age-related increases in hippocampal glial fibrillary acidic protein mRNA. *Neurobiol Aging* 18, 523 – 6.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* 20, 9104 – 10.

Mariani E, Polidori MC, Cherubini A, Mecocci P. (2005). Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview. *J Chromatogr B Analyt Technol Biomed Life Sci* 827, 65 – 75.

Mascarucci P, Taub D, Saccani S, Paloma MA, Dawson H, Roth GS, Lane MA, Ingram DK. (2002). Cytokine responses in young and old rhesus monkeys: effect of caloric restriction. *J Interferon Cytokine Res* 22, 565 – 71.

Masoro EJ, Yu BP, Bertrand HA. (1982). Action of food restriction in delaying the aging process. *Proc Natl Acad Sci U S A* 79, 4239 – 41.

Masoro EJ (1985). Aging and nutrition–can diet affect life span? *Trans Assoc Life Insur Med Dir Am* 67, 30 – 44.

Masoro EJ, McCarter RJ, Katz MS, McMahan CA. (1992). Dietary restriction alters characteristics of glucose fuel use. *J Gerontol* 47, *B*202 – 8.

Masoro EJ. (1993). Dietary restriction and aging. *J Am Geriatr Soc* 41, 994 – 9.

Masoro EJ. (1998). Influence of caloric intake on aging and on the response to stressors. *J Toxicol Environ Health B Crit Rev* 1, 243 – 57.

Masoro EJ. (2001). Physiology of aging. *Int J Sport Nutr Exerc Metab* 11, 218 – 22.

Maswood N, Young J, Tilmont E, Zhang Z, Gash DM, Gerhardt GA, Grondin R, Roth GS, Mattison J, Lane MA, Carson RE, Cohen RM, Mouton PR, Quigley C, Mattson MP, Ingram DK. (2004). Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. *Proc Natl Acad Sci U S A* 101, 18171 – 6.

Mataga N, Nagai N, Hensch TK. (2002). Permissive proteolytic activity for visual cortical plasticity. *Proc Natl Acad Sci U S A* 99, 7717 – 21.

Mataga N, Mizuguchi Y, Hensch TK. (2004). Experience-dependent pruning of dendritic spines in visual cortex by tissue plasminogen activator. *Neuron* 44, 1031 – 41.

Mattiasson G, Shamloo M, Gido G, Mathi K, Tomasevic G, Yi S, Warden CH, Castilho RF, Melcher T, Gonzalez-Zulueta M, Nikolich K, Wieloch T.

(2003). Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma. *Nat Med* 9, 1062 – 8.

Mattson MP, Chan SL, Duan W. (2002). Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. *Physiol Rev* 82, 637 – 72.

Mattson MP, Duan W, Guo Z. (2003). Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. *J Neurochem* 84, 417 – 31.

Maya Vetencourt JF, Sale A, Viegi A, Baroncelli L, De Pasquale R, O'Leary OF, Castrén E, Maffei L. (2008). The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science* 320, 385 – 8.

Mayeux R, Tang MX, Jacobs DM, Manly J, Bell K, Merchant C, Small SA, Stern Y, Wisniewski HM, Mehta PD. (1999). Plasma amyloid beta-peptide 1-42 and incipient Alzheimer's disease. *Ann Neurol* 46, 412 – 6.

McCarter R, Masoro EJ, Yu BP. (1985). Does food restriction retard aging by reducing the metabolic rate? *Am J Physiol* 248, *E*488 – 90.

McCay CM, Crowell MF, Maynard LA. (1935). The effect of retarded growth upon the length of life span and upon the ultimate body size. *J Nutr* 10, 63 – 79.

McEwen BS. (2001). Invited review: Estrogens effects on the brain: multiple sites and molecular mechanisms. *J Appl Physiol* 91, 2785 – 801.

McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM. (2005). Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. *Science* 309, 2222 – 6.

Means LW, Higgins JL, Fernandez TJ. (1993). Mid-life onset of dietary restriction extends life and prolongs cognitive functioning. *Physiol Behav* 54, 503 – 8.

Meier U, Gressner AM. (2004). Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem* 50, 1511 – 25.

Mellor J. (2006). Dynamic nucleosomes and gene transcription. *Trends Genet* 22, 320 – 9.

Merry BJ. (2004). Oxidative stress and mitochondrial function with aging–the effects of calorie restriction. *Aging Cell* 3, 7 – 12.

Merry BJ. (2002). Molecular mechanisms linking calorie restriction and longevity. *Int J Biochem Cell Biol* 34, 1340 – 54.

Mistlberger RE. (1994). Circadian food-anticipatory activity: formal models and physiological mechanisms. *Neurosci Biobehav Rev* 18, 171 – 95.

Mitchell GA, Kassovska-Bratinova S, Boukaftane Y, Robert MF, Wang SP, Ashmarina L, Lambert M, Lapierre P, Potier E. (1995). Medical aspects of ketone body metabolism. *Clin Invest Med* 18, 193 – 216.

Moreira PI, Cardoso SM, Santos MS, Oliveira CR. (2006). The key role of mitochondria in Alzheimer's disease. *J Alzheimers Dis* 9, 101 – 10.

Morishita H, Hensch TK. (2008). Critical period revisited: impact on vision. *Curr Opin Neurobiol* 18, 101 – 7.

Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, Bultsma Y, McBurney M, Guarente L. (2004). Mammalian SIRT1 represses forkhead tran-

scription factors. *Cell* 116, 551 – 63.

Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. *Nature* 424, 277 – 83.

Musi N, Fujii N, Hirshman MF, Ekberg I, Fröberg S, Ljungqvist O, Thorell A, Goodyear LJ. (2001). AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes during exercise. *Diabetes* 50, 921 – 7.

Naka F, Shiga T, Yaguchi M, Okado N. (2002). An enriched environment increases noradrenaline concentration in the mouse brain. *Brain Res* 924, 124 – 6.

Nakae J, Kitamura T, Silver DL, Accili D. (2001). The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. *J Clin Invest* 108, 1359 – 67.

Nemoto S, Fergusson MM, Finkel T. (2004). Nutrient availability regulates SIRT1 through a forkhead-dependent pathway. *Science* 306, 2105 – 8.

Nestler EJ. (1998). Antidepressant treatments in the 21st century. *Biol Psichiatry* 44, 526 – 33.

Nguyen PV, Marin L, Atwood HL. (1997). Synaptic physiology and mitochondrial function in crayfish tonic and phasic motor neurons. *J Neurophysiol* 78, 281 – 94.

Nibuya M, Morinobu S, Duman RS. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 15, 7539 – 47.

Nicholls DG, Vesce S, Kirk L, Chalmers S. (2004). Interactions between mi-

tochondrial bioenergetics and cytoplasmic calcium in cultured cerebellar granule cells. *Cell Calcium* 34, 407 – 24.

Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS. (1999). Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J Neurobiol* 39, 569 – 78.

Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO. (2005). Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310, 314 – 7.

Nithianantharajah J, Hannan AJ. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat Rev Neurosci* 7, 697 – 709.

Oh SW, Mukhopadhyay A, Svrzikapa N, Jiang F, Davis RJ, Tissenbaum HA. (2005). JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc Natl Acad Sci U S A* 102, 4494 – 9.

Oray, S., Majewska, A. and Sur, M. (2004). Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation. *Neuron* 44, 1021 – 30.

Pajvani UB, Scherer PE. (2003). Adiponectin: systemic contributor to insulin sensitivity. *Curr Diab Rep* 3, 207 – 13.

Pantaloni D, Le Clainche C, Carlier MF. (2001). Mechanism of actin-based motility. *Science* 292, 1502 – 6.

Parker JA, Arango M, Abderrahmane S, Lambert E, Tourette C, Catoire H, Néri C. (2005). Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. *Nat Genet* 37, 349 – 50.

Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci* 17, 3727 – 38.

Patel NV, Finch CE. (2002). The glucocorticoid paradox of caloric restriction in slowing brain aging. *Neurobiol Aging* 23, 707 – 17.

Pfister KK. (1999). Cytoplasmic dynein and microtubule transport in the axon: the action connection. *Mol Neurobiol* 20, 81 – 91.

Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, Leid M, McBurney MW, Guarente L. (2001). Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature* 429, 771 – 6.

Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J. W. and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. *Science* 298, 1248 – 51.

Pizzorusso T, Medini P, Landi S, Baldini S, Berardi N, Maffei L. (2006). Structural and functional recovery from early monocular deprivation in adult rats. *Proc Natl Acad Sci U S A* 103, 8517 – 22.

Plunet WT, Streijger F, Lam CK, Lee JH, Liu J, Tetzlaff W. (2008). Dietary restriction started after spinal cord injury improves functional recovery. *Exp Neurol* 213, 28 – 35.

Pollard TD, Blanchoin L, Mullins RD. (2000). Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu Rev Biophys Biomol Struct* 29, 545 – 76.

Polley DB, Kvasnák E, Frostig RD. (2004). Naturalistic experience transforms sensory maps in the adult cortex of caged animals. *Nature* 429, 67 – 71.

Porciatti V, Pizzorusso T, Maffei L. (1999). The visual physiology of the wild type mouse determined with pattern VEPs. *Vision Res* 39, 3071 – 81.

Poynter ME, Daynes RA. (1998). Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappaB signaling, and reduces inflammatory cytokine production in aging. *J Biol Chem* 273, 32833 – 41.

Putignano E, Lonetti G, Cancedda L, Ratto G, Costa M, Maffei L, Pizzorusso T. (2007). Developmental downregulation of histone posttranslational modifications regulates visual cortical plasticity. *Neuron* 53, 747 – 59.

Prusky GT, West PW, Douglas RM. (2000). Experience-dependent plasticity of visual acuity in rats. *Eur J Neurosci* 12, 3781 – 6.

Prusky GT, Douglas RM. (2003). Developmental plasticity of mouse visual acuity. *Eur J Neurosci* 17, 167 – 73.

Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92, 167 – 73.

Puigserver P, Spiegelman BM. (2001). Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 24, 78 – 90.

Rampon C, Jiang CH, Dong H, Tang YP, Lockhart DJ, Schultz PG, Tsien JZ, Hu Y. (2000). Effects of environmental enrichment on gene expression in the brain. *Proc Natl Acad Sci U S A* 97, 12880 − 4.

Rankin JW, Shute M, Heffron SP, Saker KE. (2006). Energy restriction but not protein source affects antioxidant capacity in athletes. *Free Radic Biol Med* 41, 1001−9.

Rasmuson S, Olsson T, Henriksson BG, Kelly PA, Holmes MC, Seckl JR, Mohammed AH. (1998). Environmental enrichment selectively increases 5-HT1A receptor mRNA expression and binding in the rat hippocampus. *Brain Res Mol Brain Res* 53, 285 − 90.

Rattan SI. (2008). Hormesis in aging. *Ageing Res Rev* 7, 63 − 78.

Ravagnan L, Gurbuxani S, Susin SA, Maisse C, Daugas E, Zamzami N, Mak T, Jäättelä M, Penninger JM, Garrido C, Kroemer G. (2001). Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nat Cell Biol* 3, 839 − 43.

Ray WJ, Ashall F, Goate AM. (1998). Molecular pathogenesis of sporadic and familial forms of Alzheimer's disease. *Mol Med Today* 4, 151 − 7.

Reddy PH. (2006). Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease. *J Neurochem* 96, 1 − 13.

Reese BE. (1988). 'Hidden lamination' in the dorsal lateral geniculate nucleus: the functional organization of this thalamic region in the rat. *Brain Res* 472, 119 − 37.

Reese BE, Cowey A. (1983). Projection lines and the ipsilateral retino-geniculate pathway in the hooded rat. *Neuroscience* 10, 1233 − 47.

Refsum H. (2001). Folate, vitamin B12 and homocysteine in relation to birth defects and pregnancy outcome. *Br J Nutr* 85, $S109 - 13$.

Renner MJ, Rosenzweig MR. (1987). The golden-mantled ground squirrel (Spermophilus lateralis) as a model for the effects of environmental enrichment in solitary animals. *Dev Psychobiol* 20, $19 - 24$.

Rine J, Herskowitz I. (1987). Four genes responsible for a position effect on expression from HML and HMR in Saccharomyces cerevisiae. *Genetics* 116, $9 - 22$.

Rosenzweig MR, Krech D, Bennett EL, Diamond MC. (1962). Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension. *J Comp Physiol Psychol* 55, $429 - 37$.

Rosenzweig MR. (1966). Environmental complexity, cerebral change, and behavior. *Am Psychol* 21, $321 - 32$.

Rosenzweig MR, Bennett EL. (1996). Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav Brain Res* 78, $57 - 65$.

Roth GS, Ingram DK, Lane MA. (1999). Calorie restriction in primates: will it work and how will we know? *J Am Geriatr Soc* 47, $896 - 903$.

Roth GS, Lane MA, Ingram DK, Mattison JA, Elahi D, Tobin JD, Muller D, Metter EJ. (2002). Biomarkers of caloric restriction may predict longevity in humans. *Science* 297, 811.

Rozas, C., Frank, H., Heynen, A. J., Morales, B., Bear, M. F. and Kirkwood, A. (2001). Developmental inhibitory gate controls the relay of activity to the superficial layers of the visual cortex. *J Neurosci* 21, $6791 - 801$.

Sabatino F, Masoro EJ, McMahan CA, Kuhn RW. (1991). Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. *J Gerontol* 46, $B171 - 9$.

Sacher GA, Duffy PH. (1979). Genetic relation of life span to metabolic rate for inbred mouse strains and their hybrids. *Fed Proc* 38, $184 - 8$.

Saghatelyan AK, Dityatev A, Schmidt S, Schuster T, Bartsch U, Schachner M. (2001). Reduced perisomatic inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the extracellular matrix glycoprotein tenascin-R. *Mol Cell Neurosci* 17, $226 - 40$.

Sale, A., Putignano, E., Cancedda, L., Landi, S., Cirulli, F., Berardi, N. and Maffei, L. (2004). Enriched environment and acceleration of visual system development. *Neuropharmacology* 47, $649 - 60$.

Sale A, Maya Vetencourt JF, Medini P, Cenni MC, Baroncelli L, De Pasquale R, Maffei L. (2007a). Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. *Nat Neurosci* 10, $679 - 81$.

Sale A, Cenni MC, Ciucci F, Putignano E, Chierzi S, Maffei L. (2007b). Maternal enrichment during pregnancy accelerates retinal development of the fetus. *PLoS One* 2, $e1160$.

Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 30, $805-9$.

Santos-Pinto FN, Luz J, Griggio MA. (2001). Energy expenditure of rats subjected to long-term food restriction. *Int J Food Sci Nutr* 52, $193 - 200$.

Sanz A, Caro P, Ibañez J, Gómez J, Gredilla R, Barja G. (2005). Dietary restriction at old age lowers mitochondrial oxygen radical production and leak at complex I and oxidative DNA damage in rat brain. *J Bioenerg Biomembr* 37, 83 – 90.

Sawtell NB, Frenkel MY, Philpot BD, Nakazawa K, Tonegawa S, Bear MF. (2003). NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. *Neuron* 38, 977 – 85.

Schulz JB, Dichgans J. (1999). Molecular pathogenesis of movement disorders: are protein aggregates a common link in neuronal degeneration? *Curr Opin Neurol* 12, 433 – 9.

Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolf PA. (2002). Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 346, 476 – 83.

Shatz CJ, Stryker MP. (1978). Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. *J Physiol* 281, 267 – 83.

Shi T, Wang F, Stieren E, Tong Q. (2005). SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. *J Biol Chem* 280, 13560 – 7.

Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci* 22, 3251 – 61.

Simons K. (2005). Amblyopia characterization, treatment, and prophylaxis. *Surv Ophthalmol* 50, 123 – 66.

Silver J, Miller JH. (2004). Regeneration beyond the glial scar. *Nat Rev Neurosci* 5, 146 – 56.

Soghomonian JJ, Martin DL. (1998). Two isoforms of glutamate decarboxylase: why? *Trends Pharmacol Sci* 19, 500 – 5.

Sohal RS, Ku HH, Agarwal S, Forster MJ, Lal H. (1994). Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech Ageing Dev* 74, 121 – 33.

Sonntag WE, Lynch CD, Cefalu WT, Ingram RL, Bennett SA, Thornton PL, Khan AS. (1999). Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals. *J Gerontol A Biol Sci Med Sci* 54, *B*521 – 38.

Spolidoro M, Sale A, Berardi N, Maffei L. (2009). Plasticity in the adult brain: lessons from the visual system. *Exp Brain Res* 192, 335 – 41.

Sprott RL. (1997). Diet and calorie restriction. *Exp Gerontol* 32, 205 – 14.

Sreekumar R, Unnikrishnan J, Fu A, Nygren J, Short KR, Schimke J, Barazzoni R, Nair KS. (2002). Effects of caloric restriction on mitochondrial function and gene transcripts in rat muscle. *Am J Physiol Endocrinol Metab* 283, *E*38 – 43.

Stamp JA, Mashoodh R, van Kampen JM, Robertson HA. (2008). Food restriction enhances peak corticosterone levels, cocaine-induced locomotor activity, and DeltaFosB expression in the nucleus accumbens of the rat. *Brain Res* 1204, 94 – 101.

Stephens JM, Lee J, Pilch PF. (1997). Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction.

J Biol Chem 272, 971 – 6.

Stewart J, Mitchell J, Kalant N. (1989). The effects of life-long food restriction on spatial memory in young and aged Fischer 344 rats measured in the eight-arm radial and the Morris water mazes. *Neurobiol Aging* 10, 669 – 75.

Stryker MP, Chapman B, Miller KD, Zahs KR. (1990). Experimental and theoretical studies of the organization of afferents to single orientation columns in visual cortex. *Cold Spring Harb Symp Quant Biol* 55, 515 – 27.

Sugden MC, Bulmer K, Gibbons GF, Holness MJ. (2001). Role of peroxisome proliferator-activated receptor-alpha in the mechanism underlying changes in renal pyruvate dehydrogenase kinase isoform 4 protein expression in starvation and after refeeding. *Arch Biochem Biophys* 395, 246 – 52.

Sullivan PG, Geiger JD, Mattson MP, Scheff SW. (2000). Dietary supplement creatine protects against traumatic brain injury. *Ann Neurol* 48, 723 – 9.

Sullivan PG, Dubé C, Dorenbos K, Steward O, Baram TZ. (2003). Mitochondrial uncoupling protein-2 protects the immature brain from excitotoxic neuronal death. *Ann Neurol* 53, 711 – 7.

Szél A, Röhlich P. (1992). Two cone types of rat retina detected by anti-visual pigment antibodies. *Exp Eye Res* 55, 47 – 52.

Tissenbaum HA, Guarente L. (2001). Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature* 410, 227 – 30.

Thurlow GA, Cooper RM. (1988). Metabolic activity in striate and extrastriate cortex in the hooded rat: contralateral and ipsilateral eye input. *J Comp Neurol* 274, 595 – 607.

Tong JJ. (2007). Mitochondrial delivery is essential for synaptic potentiation. *Biol Bull* 212, 169 – 75.

Tontonoz P, Hu E, Spiegelman BM. (1994). Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* 79, 1147 – 56.

Trachtenberg, J. T., Trepel, C. and Stryker, M. P. (2000). Rapid extragranular plasticity in the absence of thalamocortical plasticity in the developing primary visual cortex. *Science* 287, 2029 – 32.

Tran H, Brunet A, Grenier JM, Datta SR, Fornace AJ Jr, DiStefano PS, Chiang LW, Greenberg ME. (2002). DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. *Science* 296, 530 – 4.

Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Hee Park S, Thompson T, Karsenty G, Bradley A, Donehower LA. (2002). p53 mutant mice that display early ageing-associated phenotypes. *Nature* 415, 45 – 53.

Valle A, Guevara R, García-Palmer FJ, Roca P, Oliver J. (2008). Caloric restriction retards the age-related decline in mitochondrial function of brown adipose tissue. *Rejuvenation Res* 11, 597 – 604.

van der Horst A, Tertoolen LG, de Vries-Smits LM, Frye RA, Medema RH, Burgering BM. (2004). FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J Biol Chem* 279, 28873 – 9.

van Praag H, Kempermann G, Gage FH. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2, 266 – 70.

van Praag H, Kempermann G, Gage FH. (2000). Neural consequences of environmental enrichment. *Nat Rev Neurosci* 1, 191 – 8.

Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, Guarente L, Weinberg RA. (2001). hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* 107, 149 – 59.

Vazquez JA, Morse EL, Adibi SA. (1985). Effect of dietary fat, carbohydrate, and protein on branched-chain amino acid catabolism during caloric restriction. *J Clin Invest* 76, 737 – 43.

Verdery RB, Ingram DK, Roth GS, Lane MA. (1997). Caloric restriction increases HDL2 levels in rhesus monkeys (Macaca mulatta). *Am J Physiol* 273, *E*714 – 9.

Vikman KS, Owe-Larsson B, Brask J, Kristensson KS, Hill RH. (2001). Interferon-gamma-induced changes in synaptic activity and AMPA receptor clustering in hippocampal cultures. *Brain Res* 896, 18 – 29.

Vousden KH, Lu X. (2002). Live or let die: the cell's response to p53. *Nat Rev Cancer* 2, 594 – 604.

Walf AA, Frye CA. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* 2, 322 – 8.

Walford RL, Mock D, Verdery R, MacCallum T. (2002). Calorie restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period. *J Gerontol A Biol Sci Med Sci* 57, *B*211 – 24.

Wan R, Camandola S, Mattson MP (2003). Intermittent food deprivation improves cardiovascular and neuroendocrine responses to stress in rats. *J Nutr* 133,

1921 – 9.

Wang MC, Bohmann D, Jasper H. (2003). JNK signaling confers tolerance to oxidative stress and extends lifespan in Drosophila. *Dev Cell* 5, 811 – 6.

Wang MC, Bohmann D, Jasper H. (2005). JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* 121, 115 – 25.

Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. (1999). Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. *Nat Genet* 23, 425 – 8.

Weeber EJ, Levy M, Sampson MJ, Anflous K, Armstrong DL, Brown SE, Sweatt JD, Craigen WJ. (2002). The role of mitochondrial porins and the permeability transition pore in learning and synaptic plasticity. *J Biol Chem* 277, 18891 – 7.

Weindruch R, Walford RL. (1982). Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. *Science* 215, 1415 – 8.

Weindruch R, Naylor PH, Goldstein AL, Walford RL. (1988). Influences of aging and dietary restriction on serum thymosin alpha 1 levels in mice. *J Gerontol* 43, *B*40 – 2.

Weindruch R, Sohal RS. (1997). Seminars in medicine of the Beth Israel Deaconess Medical Center. Caloric intake and aging. *N Engl J Med* 337, 986 – 94.

Weindruch R, Kayo T, Lee CK, Prolla TA. (2002). Gene expression profiling of aging using DNA microarrays. *Mech Ageing Dev* 123, 177 – 93.

Wheeler MD, Smutney OM, Check JF, Rusyn I, Schulte-Hermann R, Thur-

man RG. (2003). Impaired Ras membrane association and activation in PPARalpha knockout mice after partial hepatectomy. *Am J Physiol Gastrointest Liver Physiol* 284, $G302 - 12$.

Wolkow CA, Kimura KD, Lee MS, Ruvkun G. (2000). Regulation of C. elegans life-span by insulinlike signaling in the nervous system. *Science* 290, $147 - 50$.

Wong G, Goldshmit Y, Turnley AM. (2004). Interferon-gamma but not TNF alpha promotes neuronal differentiation and neurite outgrowth of murine adult neural stem cells. *Exp Neurol* 187, $171 - 7$.

Workman JL. (2006). Nucleosome displacement in transcription. *Genes Dev* 20, $2009 - 17$.

Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R, Goldstein BJ. (2003). Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes* 52, $1355 - 63$.

Yeagley D, Guo S, Unterman T, Quinn PG. (2001). Gene- and activation-specific mechanisms for insulin inhibition of basal and glucocorticoid-induced insulin-like growth factor binding protein-1 and phosphoenolpyruvate carboxykinase transcription. Roles of forkhead and insulin response sequences. *J Biol Chem* 276, $33705 - 10$.

Yeung JM, Friedman E. (1991). Effect of aging and diet restriction on monoamines and amino acids in cerebral cortex of Fischer-344 rats. *Growth Dev Aging* 55, $275 - 83$.

Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW. (2004). Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 23, $2369 - 80$.

You H, Jang Y, You-Ten AI, Okada H, Liepa J, Wakeham A, Zaugg K,

Mak TW. (2004). p53-dependent inhibition of FKHRL1 in response to DNA damage through protein kinase SGK1. *Proc Natl Acad Sci U S A* 101, 14057 – 62.

Youdim KA, Joseph JA. (2001). A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free Radic Biol Med* 30, 583 – 94.

Yu Z, Luo H, Fu W, Mattson MP. (1999). The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. *Exp Neurol* 155, 302 – 14.

Yu ZF, Mattson MP. (1999). Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. *J Neurosci Res* 15, 830 – 9.

Yudkoff M, Daikhin Y, Nissim I, Lazarow A, Nissim I. (2001). Ketogenic diet, amino acid metabolism, and seizure control. *J Neurosci Res* 66, 931 – 40.

Zangarelli A, Chanseaume E, Morio B, Brugère C, Mosoni L, Rousset P, Giraudet C, Patrac V, Gachon P, Boirie Y, Walrand S. (2006). Synergistic effects of caloric restriction with maintained protein intake on skeletal muscle performance in 21-month-old rats: a mitochondria-mediated pathway. *FASEB J* 20, 2439 – 50.

Zeisel SH. (1997). Choline: essential for brain development and function. *Adv Pediatr* 44, 263 – 95.

Zhao Y, Sun H, Lu J, Li X, Chen X, Tao D, Huang W, Huang B. (2005). Lifespan extension and elevated hsp gene expression in Drosophila caused by histone deacetylase inhibitors. *J Exp Biol* 208, 697 – 705.

Zhu H, Guo Q, Mattson MP. (1999). Dietary restriction protects hippocampal neurons against the death-promoting action of a presenilin-1 mutation. *Brain Res* 842, 224 – 9.

Zhu M, de Cabo R, Lane MA, Ingram DK. (2004). Caloric restriction modulates early events in insulin signaling in liver and skeletal muscle of rat. *Ann N Y Acad Sci* 1019, 448 – 52.