Modular organization of motor behavior in the frog's spinal cord

Emilio Bizzi, Simon F. Giszter, Eric Loeb, Ferdinando A. Mussa-Ivaldi and Philippe Saltiel

The complex issue of translating the planning of arm movements into muscle forces is discussed in relation to the recent discovery of structures in the spinal cord. These structures contain circuitry that, when activated, produce precisely balanced contractions in groups of muscles. These synergistic contractions generate forces that direct the limb toward an equilibrium point in space. Remarkably, the force outputs, produced by activating different spinal-cord structures, sum vectorially. This vectorial combination of motor outputs might be a mechanism for producing a vast repertoire of motor behaviors in a simple manner.

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Emilio Bizzi, Eric Loeb and Philippe Saltiel are at the Dept of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139-4307, USA, Simon F. Giszter is at the Dept of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129, USA, and Ferdinando A. Mussa-Ivaldi is at the Dept of Physiology and the Dept of Physical Medicine and Rehabilitation, Northwestern University Medical School, Chicago, IL 60611-3008, USA.

TO SPECIFY A PLAN of action, the CNS must first transfer sensory inputs into motor goals, such as the direction, amplitude and velocity of the intended movement. Then, to execute movements, the CNS must convert these desired goals into signals that control the muscles that are active during the execution of even the simplest kind of limb trajectory. Thus, the CNS must transform information about a small number of variables (direction, amplitude and velocity) into a large number of signals to many muscles. Any transformation of this type is 'ill-posed' in the sense that an exact solution might be either not available or not unique. How the nervous system computes these transformations has been the focus of recent studies[1–5].

Recently, a map in the spinal cord that might represent a mechanism whereby the CNS performs a transformation of motor goals into the muscle synergies that subserve a limb's movement has been discovered. Electrical and chemical (NMDA) stimulation of this interneuronal circuitry has been shown to impose a specific balance of muscle activation, leading to a convergent force field (CFF). These synergistic contractions generate a finite number of force patterns that direct the limb toward an equilibrium point in space.

A second observation, derived from microstimulation of the frog's spinal cord, is that the fields that are induced by the focal activation of the cord follow a principle of vector summation. Simultaneous stimulation of two sites, each generating a different force field, results in the vector sum of the two fields. This observation has led to a novel hypothesis for explaining movement and posture based on combinations of a few basic modules. This finite set of force patterns might be viewed as representing an elementary alphabet from which, through superimposition, a vast number of actions could be fashioned by impulses that are conveyed by supraspinal pathways.

Convergent force fields (CFFs)

Microstimulation of the premotoneuronal circuitry of the spinal cord produces precisely balanced contractions in groups of muscles[6–8]. These synergistic contractions generate forces that direct the limb toward an equilibrium point in space. To measure the mechanical responses of the activated muscles, Bizzi and colleagues[6] attached the right ankle of a frog to a six-axis force transducer, as shown in Fig. 1 (Ref. 7). The output of the transducer was a set of three forces and three torques. However, generally, measurements were made in only two dimensions (x and y). Only in a few instances were three-dimensional convergent fields recorded[9]. At each ankle location, the net-force vector, obtained in response to stimulation, was expressed as the sum of two components – the 'resting'-force vector and the 'active'-force vector. The resting-force vector corresponded to the force measured before the onset of the stimulus. The active-force vector represented the additional force that was induced by the electrical or chemical stimulation.

To record the spatial variations of forces that are generated by the leg muscles, Bizzi and colleagues[6] and Giszter and colleagues[7] followed a three-part procedure on 250 frogs. First, they placed the frog's leg at a location within the leg's workspace. Second, they stimulated a site in the spinal cord, and recorded the direction and amplitude of the elicited isometric force at the ankle. Third, they repeated the stimulation procedure with the ankle placed at each of nine to 16 locations. These locations sampled a large portion of the region of the horizontal plane that could be reached by the frog's ankle (that is, the leg's workspace). At each location, they stimulated the same site in the spinal cord and recorded the force vector, $F = (F_x, F_y)$. Although the site of spinal-cord stimulation was constant throughout, Bizzi and colleagues[6] found that the elicited force vector varied as they placed the leg at different locations. These changes in force throughout the workspace resulted from a variety of mechanical factors, such as the length, moment arms and viscoelastic properties of the muscles. In addition, reflex modulation of the muscles' activations played a role in the development of the forces at the ankle.

The collection of the measured forces corresponded to a total force field[7] (Fig. 1). In most instances (80%), the spatial variation of the measured force vectors resulted in a field that was at all times both convergent and characterized by a single equilibrium point (that is, a point at which the amplitude of the F_x and F_y force components was zero). This equilibrium point represented the locus at which the leg would have been at steady state if it was free to move. That is, the equilibrium point was the position at which there would be no endpoint force. The interpretation of the force-field data was simplified when the resting forces were removed. Giszter and colleagues[7] found that the active-field data fell into a few classes or force-field types (Fig. 2).

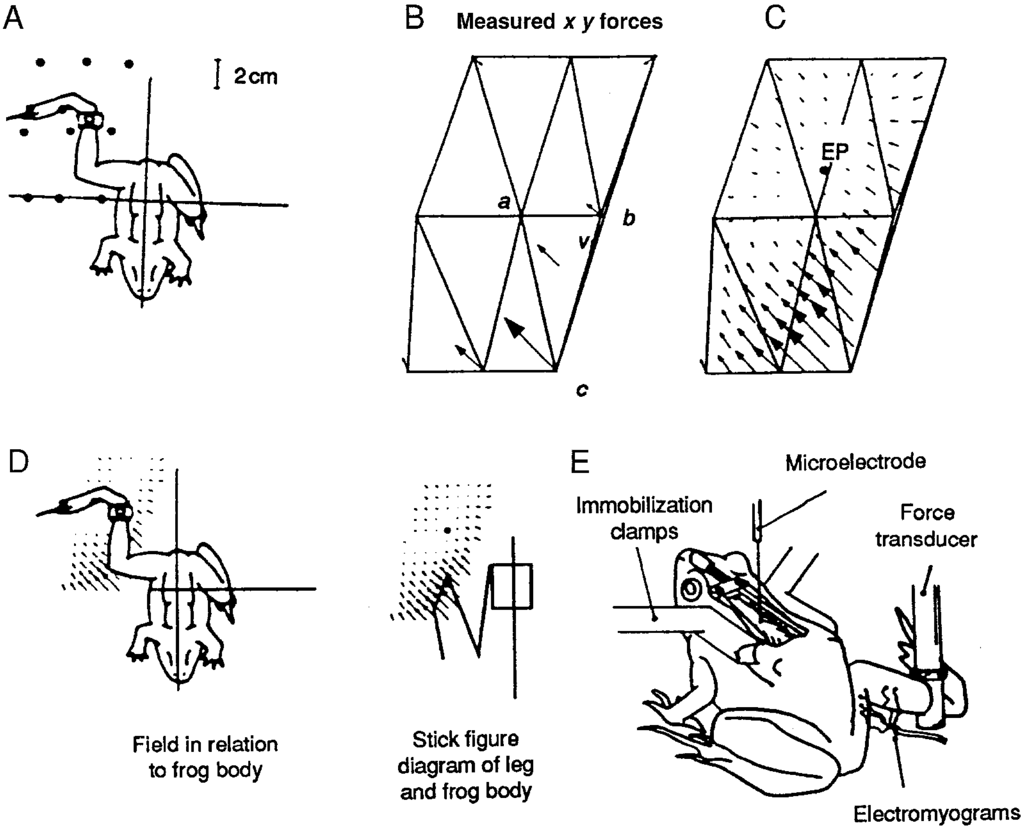

Fig. 1. *The apparatus and method of construction of a force field.* **(A)** *A collection of forces is recorded at several different spatial locations (circles).* **(B)** *A minimum perimeter (Delaunay) tessellation of the nine points is constructed. Within each triangle, vectors are estimated using an exact linear interpolation based on the three corner vectors. Thus, vector v is calculated using vectors at vertices a, b and c.* **(C)** *The interpolated field is used to find any equilibria (EP).* **(D)** **(Left.)** *The interpolated force field is shown in relation to the frog in the apparatus.* **(Right.)** *This is reduced to a stylized construction that is used to express the relationship of frog body axis and leg to the interpolated force field in the remaining figures.* **(E)** *The apparatus. The spine is clamped, and the pelvis is held clamped by restraints (not shown). The stimulating electrode is in the spinal gray. The mechanical response to stimulation is recorded at the force sensor that is attached to the limb at the ankle. The limb configuration is constrained fully by the pelvis restraint and the force sensor. The activation of the leg's muscles was elicited by microstimulating the lateral and intermediate neuropil zone (300–1000 μm in depth and 200–500 μm from the midline). Each stimulus consisted of a train of anodal current impulses. Typically, the train lasted 300 ms. The peak current's amplitude ranged between 1 μA and 8 μA. This peak magnitude corresponded to an estimated stimulation volume of less than 100 μm radius.* Modified from Ref. 7.

Different groups of leg muscles were activated as the stimulating electrodes were moved to different loci of the lumbar spinal cord in the rostrocaudal and mediolateral direction. After mapping most of the premotor area of the lumbar cord, Bizzi and colleagues[6] and Giszter and colleagues[7] reached the conclusion that there were at least four areas, and that a distinct type of CFF was elicited from each of the four spinal regions. These regions formed stripes that were oriented rostrocaudally. The regions extended dorsoventrally over a distance of at least 300 μm in depth. Within each region, a qualitatively similar set of *x* and *y* forces were produced. This map of active fields is shown in Fig. 2.

Vector summation of CFF modules: co-stimulation of two sites in the spinal cord

The fields that are induced by the focal activation of the spinal cord follow a principle of vectorial summation (Fig. 3). Specifically, vectorial summation was investigated in the following experimental paradigm. First, a focal electrical stimulation was delivered to a site, A, of the spinal cord and the subsequent active field $\Phi_A(x)$ was determined. Following the measurement of $\Phi_A(x)$, a second electrical stimulation was applied to a different site, B, and a field $\Phi_B(x)$ was derived. From these two fields, their sum, $\Phi_\Sigma(x) = \Phi_A(x) + \Phi_B(x)$, was computed at all the sampled points, $\{x_1, x_2, \dots x_k\}$. Finally, another active field, $\Phi_\&(x)$, was measured by stimulating the sites A and B simultaneously. When Mussa-Ivaldi and colleagues[10] compared the 'co-stimulation fields', $\Phi_\&(x)$, with the corresponding 'summation fields', $\Phi_\Sigma(x)$, they found that in more than 87% of cases, the two fields were equivalent[10].

Vector summation of force fields implies that the complex non-linearities that characterize the interactions both among neurons and between neurons and muscles are in some way eliminated. More importantly, this result has led to a novel hypothesis for explaining movement and posture based on combinations of a few basic elements. The few active-force fields that are stored in the spinal cord might be viewed as representing motor primitives from which, through superimposition, a vast number of movements can be produced by impulses that are conveyed by supraspinal pathways. Through mathematical modeling, Mussa-Ivaldi[11], along with Giszter[12], has verified that this novel view of the generation of movement and posture has the 'competence' that is required for controlling a wide repertoire of motor behaviors.

The mechanical basis of force-field summation: co-stimulation of muscles and kinematic redundancy

Individual muscles whose afferent and efferent connections had been severed were stimulated electrically. Muscle forces (*x* and *y*) were recorded while a frog's ankle was placed at each of nine locations in the workspace. When the limb was connected to the force sensor above the ankle, sum and co-stimulation force fields of different muscles displayed a high degree of similarity. In this experimental condition, the position of the sensor was related to the joint angles of the leg in a 'non-redundant' configuration, that is, once the sensor position was fixed, the joint angles were determined uniquely. It is simple to prove mathematically[13] that, in this case, the force fields that are generated by two independent actuators (such as two separate leg muscles) sum vectorially.

However, we were surprised to observe endpoint-field summation also when the frog's leg was connected to

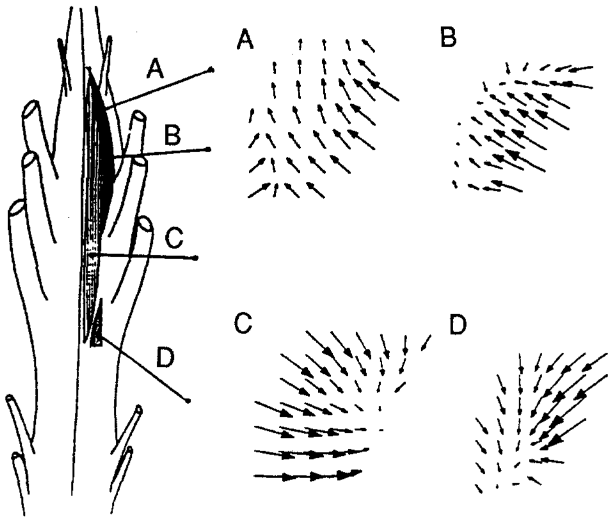

Fig. 2. ***Convergent force fields (CFFs).*** **(Left.)** *Regions of the lumbar spinal cord that contain the neural circuitry that specifies the force fields (*A–D*). Within each region, similar sets of CFFs are produced. The diagram is based on 40 CFFs that were elicited by microstimulation of premotor regions in three frogs with transected spinal cords.* **(Right.)** *Four types of CFFs. To facilitate comparison among CFFs that were recorded in different animals, the passive force field was subtracted from the force field that was obtained in the steady state.* Reproduced from Ref. 6.

the force sensor in a redundant configuration[10]. In these experiments, the foot was attached to the sensor through a gimbal that enabled the leg to assume a variety of configurations while the center of the foot was held at a fixed location of the plane. Because six joint angles (three at the hip, one at the knee, and two at the ankle) were needed to define the configuration of the frog's leg, each endpoint position did not define the limb configuration uniquely, which instead varied with the particular muscle or muscles that were activated. It is easy to demonstrate that, in this redundant case, the forces might not combine according to vector summation when independent muscles are co-activated[13]. However, vector summation in specific cases cannot be excluded. In fact, the surprising experimental result of the muscles' co-stimulation of the redundant limb indicated that vectorial summation is the predominant rule, describing the effect of combining two stimuli[10].

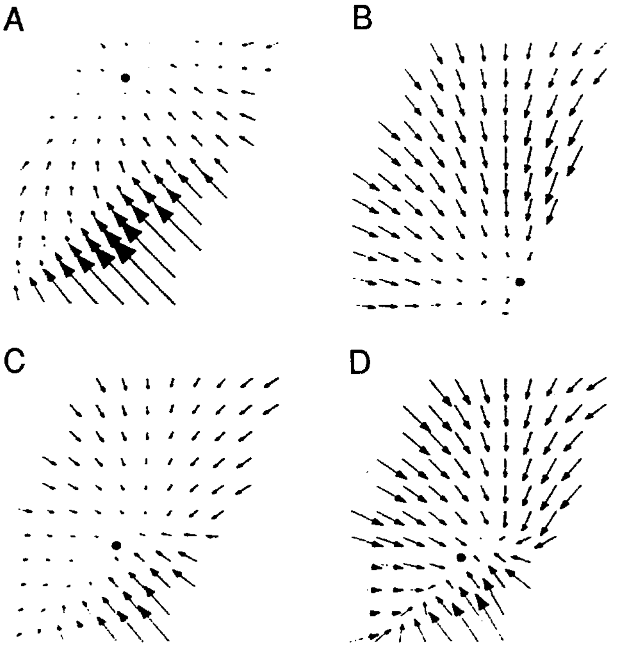

Fig. 3. ***Combinations of multiple electrical stimuli.*** **(A)** *and* **(B)** *The individual fields that result from electrical stimulation at two different sites in the premotor areas of the lumbar spinal cord. The equilibrium of field A is an extension, and B is in flexion.* **(C)** *The computed field (ab) is predicted by a simple vectorial summation of fields* A *and* B. **(D)** *The field that is elicited by stimulation of* A *and* B *together. The equilibrium point is indicated by a filled circle. The degree of similarity between the summation* (C) *and the co-stimulation* (D) *fields was quantified by a spatial correlation coefficient. In 87.8% of co-stimulations, the correlation coefficient was greater than 0.9. A threshold of 0.9, for deciding whether two fields were similar, reflected the level of variability that was inherent in the experimental procedure that was used for measuring the field.* Reproduced from Ref. 6.

Temporal evolution of the force fields

In general, the activation of a region within the spinal cord does not produce a fixed force field. Instead, following the onset of a stimulation, the force vector that is measured at each limb location changes continuously with time[7]. Therefore, the mechanical outcome of neural activation can be described as a force field that depends continuously upon both space and time. This time-varying field is the expression of a mechanical wave that summarizes the combined action of the muscles that are affected by the stimulation. Mechanical waves of the same kind can be used to describe the operation of central pattern generators and of other natural structures that are involved in the control of motor behavior. An important element in this description is the location at which the force vector vanishes. This location is called an 'equilibrium point'. At all latencies after the onset of a stimulation, the force field converges toward an equilibrium position. As the force vectors that are elicited by a stimulus change continuously in time so does the equilibrium position: the sites that are occupied by the equilibrium position at subsequent points of times define a spatial trajectory.

On the basis of experimental observations on deafferented monkeys, Bizzi and colleagues[14] concluded that even the simplest reaching movements are not generated by a sudden transition from initial to final posture. Instead, a limb is driven toward the target by a smooth temporal sequence of stable attractors. This temporal sequence has been termed a 'virtual trajectory'[15]. The sequence of stable equilibrium points that follow a spinal microstimulation constitutes a virtual trajectory and, indeed, the stimulation experiments in the spinal frog are regarded as the first direct evidence that a virtual trajectory is the product of neural activations in the motor system.

Control experiments

The results that are described in this review indicate that stimulation of the premotoneuronal circuitry of the spinal cord imposes, a specific balance of muscle activation that leads to a CCF. Giszter and colleagues[7] and Loeb and colleagues[16] have shown that this pattern of forces is not established by a random activation of motoneurons, nor from an activation of the fibers of passage of the descending and sensory systems. Giszter and colleagues have, in fact, shown that in chronically transected frogs that were kept alive for up to six weeks before performing microstimulation, the convergence of force fields was essentially unchanged[7]. Loeb and colleagues demonstrated a similarity of force-field patterns both before and after acute deafferentation[16], and in chronically deafferented frogs.

In addition to the results that were obtained by deafferentation and transection, there are other experiments that contribute to our understanding of the circuitry that subserves the expression of distinct force fields. For example, Saltiel and Bizzi[17] found that the force patterns that were produced by NMDA, which was applied microiontophoretically to the spinal cord, matched those elicited electrically in 68% of cases. This excludes the possibility that the force fields derive from the stimulation of axons of descending, ascending and reflex pathways, and points to the various types of cells of the spinal cord as key elements in the production of force fields. While there is a variety of cell types in the spinal cord, we were able to rule out the motoneurons as the source of the force fields by the experiments described below.

When microelectrodes were placed directly among the motoneurons' somas or in the ventral roots, force fields with divergent or parallel patterns rather than convergent patterns were often obtained. However, because force fields were obtained from the middle third of the spinal gray where stimulation might activate the dendrites of the motoneurons randomly, a set of experiments that was based on recording the force field of individual leg muscles, in order to rule out the direct stimulation of motoneurons, was designed. To test the origins of CFFs, the mechanical responses that were induced by the electrical stimulation of individual muscles were measured. Each muscle was stimulated through an implanted pair of EMG electrodes, and individual muscle force fields from 11 leg muscles, which represents more than 90% of the total muscle mass acting on the leg were collected. Next, random combinations of the 11 measured muscle fields were simulated, and each measured field was modulated by multiplying it with a randomly selected coefficient that represented a level of muscle activation. Sets of combined fields were obtained by adding together all these modulated fields. This computation simulated a random combination of muscle activations that generated force fields without any afferent control.

Over a set of 20 000 simulated combinations, only 8.4% of fields had an equilibrium point within the tested workspace. Thus, random recruitment of motoneurons could not account for the CFFs that were observed in the majority of our experiments. In addition, our simulation indicated that the frog's leg muscles constitute a mechanical system that is biased toward extension: their random combination tended to produce mostly extension forces. In contrast, stimulation of the premotor layers in the spinal cord resulted in the generation of equilibrium points that were located predominantly within the workspace. Thus, these results suggest that the neural circuits of the spinal cord are organized to compensate for the structural imbalance of the musculo-skeletal system. A second question that we addressed was whether the motoneuron topography within the spinal cord might account for the force fields that are observed. Motoneurons have distinct distributions in the rostrocaudal dimension of the cord. To test the importance of topography, muscle force field was combined in proportion to the estimated motoneuron densities in each region of the spinal cord. The pattern of force-field variations that was predicted by this simulation was similar to the results of direct stimulation in the motor nuclei but did not account for the results of stimulation of the premotor areas.

In summary, the results that were derived from electrical or chemical activation of the spinal cord, in conjunction with the control experiments, indicate that activations of muscles cannot be established by random activation of motoneurons, nor by the activation of the fibers of passage that belong to the sensory and descending systems. On the basis of these control experiments, we concluded that a population of interneurons, yet to be determined, must be the source of the specific types of CFFs.

Concluding remarks

The presence of a few modules in the lumbar spinal cord that produce precisely balanced contractions of groups of muscles have been identified, and a few regions of the spinal cord from which these contractions are elicited have been outlined. Recent work in the owl, the frog and the turtle is consistent with the idea of modularity. In the owl, Masino and Knudsen[18] have shown that orienting head saccades can be understood as due to the action of orthogonal saccade generators that can be activated separately. Each saccade generator must control many degrees of freedom in the owl neck in a sophisticated way in order to provide the apparent simplicity of control. In the frog, Masino and Grobstein[19] have shown that the control of azimuth and strike distance is located in separate pathways that descend into spinal cord. In the turtle, Berkowitz and Stein[20–22] showed that the kinematic motions and muscle activity of scratch and wipe movements could be decomposed into a set of phases. In turtles, these phases could be connected in different orders, and in different patterns, to blend some types of scratch.

Research into the motor functions of higher vertebrates is also relevant to modularity. Alstermark and collaborators[23–25] have identified populations of propriospinal interneurons in the C3–C4 segments of cat, which project to cells in laminae VI, VII, VIII and IX of the cat spinal cord. Their studies show that these interneurons act to co-ordinate activation and use of spindle feedback across multiple joints and among complex muscle synergies. With regard to these propriospinal systems, Jankowska and Edgley[26] suggested that these interneurons might be used not in one particular type of movement but in a variety of movements, and contribute to postural reactions and locomotion, as well as to various segmental reflexes and centrally initiated movements. Finally, recent field-potential studies have identified, in mammals, regions of cord that are particularly active following activation from the mesencephalic locomotor region[27]. These data are consistent with the idea of regional localization of some interneuronal systems in specific areas of the spinal gray.

Clearly, specialized modules for the control of posture and movement have been found in a number of vertebrate species. Our experiments contribute to this body of evidence and provide a new perspective on how the endpoint forces that are generated by stimulation of individual interneuronal zones combine to produce complex motor behavior. These zones are the likely targets of supraspinal influences that are conveyed by pyramidal axons, which are known to branch extensively in the spinal cord[28]. These divergent fibers, which convey signals specifying the direction of the hand in space[29,30], could possibly form the basis for transferring the direction of hand movements into a temporal sequence of force fields.

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Selective neuronal vulnerability in the hippocampus – a role for gene expression?

Steven S. Schreiber and Michel Baudry

Proposed mechanisms of neurodegeneration focus generally on the triggering of toxic biochemical pathways by an increased intracellular concentration of Ca^{2+}. Recent evidence also suggests that Ca^{2+} causes transcriptional activation of so-called 'cell-death genes'. Efforts to elucidate the basis of selective vulnerability have relied on animal models of delayed neuronal death in the hippocampus. Biochemical and morphological data indicate that delayed neuronal death is a form of programmed cell death, or apoptosis. Observations that specific genes are activated transcriptionally for prolonged times in neuronal populations that are undergoing delayed death suggest that active gene expression is part of the neuronal-death cascade. Although a direct causal role remains to be proven, evidence implicates certain genes in neuronal-death pathways.

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Steven S. Schreiber is at the Dept of Neurology, University of Southern California, School of Medicine, 2025 Zonal Avenue, MCH 142, Los Angeles, CA 90033, USA, and Michel Baudry is at the Neuroscience Program, University of Southern California, Los Angeles, CA 90089-0191, USA.

DESPITE RECENT SCIENTIFIC advances, the mechanism(s) that target specific neuronal populations for degeneration in human-brain diseases remains unknown. Selective neuronal vulnerability is a feature of neurodegenerative diseases, certain types of epilepsy and hypoxic–ischemic injury[1]. Understanding the biochemical basis of neuronal vulnerability, therefore, could provide insight into the pathogenesis of some of the major causes of neurological morbidity and mortality across a wide range of age groups.

The highly organized laminar structure of the mammalian hippocampus, which is one of the most frequently targeted regions, offers a unique opportunity to study selective vulnerability using animal models of neurodegeneration. For example, both status epilepticus that is induced by kainic acid, and transient global ischemia, result in delayed forms of neuronal death that selectively destroy hippocampal fields CA3/CA1 and CA1, respectively[2,3]. The results of investigations that use animal models of delayed neuronal death suggest that activation of genes contributes to the pathophysiology of selective neuronal injury.

The role of Ca^{2+} in neuronal-death pathways

Accumulating evidence supports the theory that neuronal death that follows insults, such as prolonged seizures or cerebral ischemia, is initiated by overactivation of glutamate receptors, and an increased intracellular concentration of Ca^{2+} (Ref. 4). Increased concentrations of Ca^{2+} can then trigger potentially lethal biochemical pathways that involve degradative enzymes (that is, proteases and endonucleases) or increased synthesis of oxygen free radicals, or both[4]. In addition, Ca^{2+} can regulate expression of genes through post-translational modification of transcription factors that bind to 5′-situated Ca^{2+}-response elements[5]. Thus, increased intracellular concentrations of Ca^{2+} could also lead to the transcriptional