4 Electrophysiological Methods: Application in Nutritional Neuroscience

*Rubem Carlos Araújo Guedes*

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4.1 INTRODUCTION

This chapter presents the main electrophysiological methods currently used to study brain function. Some data obtained with such methods are reported and discussed. Techniques such as the recording of spontaneous activity [electroencephalogram (EEG)] and of sensory-evoked potentials are described. The technical principles of each electrophysiological method are presented in a lucid manner, allowing nonspecialists to read the text easily, using it for a better understanding of how the brain develops and functions under a variety of nutritional conditions.

Particular attention is devoted to the electrophysiological recording of the phenomenon known as spreading depression (SD) of brain electrical activity. SD is an interesting response presented by the brain tissue as a consequence of electrical, mechanical, or chemical stimulation of one point of its surface. Changes in SD electrical features associated with nutritional conditions have been studied by my laboratory over the last two decades and some interesting data are presented and discussed to illustrate how electrophysiological methods can be applied in nutritional neuroscience.

First, a brief account is presented on the relationship between nutritional alterations and brain function. Also, the general principles for the use of electrophysiological techniques in studies involving brain function are briefly described. Then the use of EEG and evoked potential recordings in nutritional neuroscience studies is discussed and, finally, my experience on the recording the phenomenon of cortical SD is detailed.

4.1.1 Nutritional Alterations and Brain Function

The deficiency of one or more nutrients in the diet can undoubtedly disrupt the biochemical and morphological organization of the brain, and this is usually followed by repercussions on its
function. Depending on the intensity and duration of the nutritional disturbances, the effects may result in deleterious consequences, which in some cases can be more or less devastating for the whole organism (Morgane et al., 1978, 1993). Basic neural functions such as processing of sensory information and perception of the corresponding sensation, as well as execution of motor tasks, can be affected to more or less extent by nutritional deficiency. This is also the case of the more elaborated functions such as those involving cognition, consciousness, emotion, learning, and memory. Disturbances of such processes can also lead to important pathological conditions (Almeida et al., 2002).

In several parts of the world malnutrition still affects an impressive number of children, and this has influenced several research groups in their decisions to investigate in laboratory animals, as well as in humans, the effects of early malnutrition on the adult central nervous system, thereby generating an extensive body of data on this subject. Similar to what has been extensively documented in animals, malnutrition in children can also have grave consequences on their development, depending on the period of occurrence and the intensity of the nutritional deficiency (Grantham-McGregor, 1995). On the other hand, although less investigated, it is currently accepted that excessive food intake can also interfere with brain development and function (Almeida et al., 2002).

Different approaches have been used to understand to what extent such nutritional disorders affect neuroanatomical, biochemical, and electrophysiological aspects of the brain. It is now clear that such changes are much more severe when malnutrition coincides with the so-called brain growth spurt, which corresponds to the highest speed of neurogenesis, gliogenesis, and cell migration, in the neural tissue (Morgane et al., 1993). Under these conditions, the electrophysiological activity can also be considerably affected in animals, both in the peripheral (Silva et al., 1987) and the central nervous system (Morgane et al., 1978, 1993). Also, reports are available describing increased susceptibility of malnourished rats to processes related to neural excitability, such as higher reactivity to aversive stimuli (Rocinholi et al., 1997) and to experimentally induced seizures (Palencia et al., 1996; Stern et al., 1974). All the evidence documenting nutritionally related changes in neural electrophysiological phenomena and in nervous excitability prompted us to investigate changes in brain electrical activity by using, as a model, the phenomenon known as SD of brain electrical activity, which is addressed in Section 4.4.

4.1.2 General Principles in Neural Electrophysiology

In all moments of its history, the living brain has continuously produced electrical signals, and this constitutes what we call the spontaneous electrical activity of the brain. "Spontaneous," in this context, should not be understood as an activity generated without a causal stimulus. It actually means that this activity is generated without the intentional stimulation of the researcher or observer; that is, the causal stimulus is endogenous. Different techniques can be used for recording this cerebral activity in laboratory animals and humans. Valuable information can be obtained with the use of techniques that record the activity produced simultaneously by a group of many thousands of neurons located in close anatomical relationship in the brain. The recording obtained in this manner, called EEG, is obtained as electric potential differences between several pairs of electrodes placed at distinct brain regions. These macroelectrodes (usually fine wire having at the tip metal discs a few millimeters in diameter) are fixed on the scalp by special pastes with high electric conducting properties. As a noninvasive technique, EEG recording is extensively used in human patients to help diagnose certain neurological diseases. In laboratory animals, the electrodes (usually of smaller dimensions, mainly fine wires without any disc at the tip) are preferably fixed (under anesthesia) throughout small holes drilled in the skull, with the electrode tips either just touching the dura mater [recording the activity of the cerebral cortex, what we call electrocorticogram (ECoG)] or deeply inserted into the brain tissue in order to record the activity of a specific subcortical structure. After electrode implantation, the animal can be allowed to recover from anesthesia, so
that the brain electrical activity can be recorded on several occasions during periods of normal awaking or sleeping states.

The EEG presents features that are quite reproducible, provided the recording conditions remain invariable. In this situation, the electrical activity produced by the cerebral cortex of a healthy adult organism displays a pattern of continuous oscillations, generating waves of electrical potential with certain frequencies and amplitudes. This pattern is currently very well known and can change depending, for example, on the brain region in which the recording is performed (e.g., frontal vs. occipital) or on the state of consciousness of the individual (whether he or she is awake or sleeping). In the awake condition, the electrical activity can change depending on whether the individual has eyes open or closed or whether he or she is mentally performing a simple arithmetic operation (e.g., multiplying two small numbers). In a sleeping person, the electrical activity changes its pattern when a superficial stage of sleep turns into a deeper one. Figure 4.1 shows examples of EEG recordings under several physiological and pathological conditions. Changes in these patterns

FIGURE 4.1 Examples of human electroencephalograms (EEGs) showing typical patterns in different physiological and pathological states. (A) Normal subject, at resting; illustrates the higher amplitudes of EEG waves at the posterior cerebral regions (alpha rhythm) as compared with the beta rhythm predominant at the anterior areas (compare the posterior traces T3-O1 and T4-O2 with the anterior ones F3-C3 and F4-C4, respectively). (B) Normal subject, at resting; illustrates EEG changes (reduction in amplitude and increase in frequency) induced by opening the eyes (during the fifth and the sixth seconds of the record, marked by the black bar). The big deflections at the upper trace are artifacts provoked by eyelid movements. (C) Normal subject, sleeping, to show the appearance of the so-called fuses of sleep (more easily identifiable at the traces marked by black bars). Note also the general pattern of waves with low frequency and high amplitudes, typical of the sleeping state. (D) Abnormal EEG, showing irritative activity (high-amplitude waves) at the temporal and frontal regions of the left hemisphere (marked by black bars). According to international conventions, even and odd numbers (at the left side of the traces) refer to the right and left hemispheres, respectively; F, P, T, and O refer to the frontal, parietal, temporal, and occipital regions of the brain, respectively; Cz, central electrode (at the vertex of the skull); Rf, reference electrode (in this case at the ears). Numbers at the superior part of the traces indicate time in seconds.
associated with nutritional alterations can be identified and studied by using the EEG technique. In laboratory animals, it is also possible, by using very fine microelectrodes (glass pipettes with tip diameters ranging from one to a few micrometers), to record the activity of a single neuron, called unit activity. Events occurring in a single neuron can be recorded intracellularly or extracellularly. Depending on the tip dimensions and geometry (as well as on the electrical impedance) of the electrode, extracellular recordings can document the activity of a single cell or of a group of few neurons (multunit activity; Figure 4.2). These techniques can be very useful to study how nerve impulse-mediated transmission of information between neurons can be influenced by nutritional variables.

In addition, the brain can react to exogenous (sensorial or electrical) stimuli applied intentionally by the researcher. The electrical activity produced in response to an exogenous stimulus is called evoked (or provoked) potential and can be recorded with either macroelectrodes (as extracellular field potentials) or microelectrodes (as intra- or extracellular unit activities). Evoked activity can be distinguished from the spontaneous one, because it is temporally associated with the exogenous stimulus and usually presents very characteristic waveforms, which differ from the spontaneous

**FIGURE 4.1** (Continued)

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EEG rhythmic oscillations. The adequate recording of these evoked responses can also represent a helpful tool to investigate the ability of the brain in reacting to incoming information reaching the brain through a specific sensory pathway. Several physiological processes in the brain, such as the wakefulness–sleep cycle, arousal, and dreaming, can be studied by EEG and evoked potential recordings. Similarly, these techniques can help diagnose some pathological states such as epilepsy, brain tumors, coma, and clinical brain death.

A more detailed analysis on some technical and theoretical aspects of such methods is beyond the scope of this chapter. The reader can find this information in several reference sources (see, e.g., Bures et al., 1976).

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### 4.2 THE ELECTROENCEPHALOGRAM (EEG) IN NUTRITIONAL NEUROSCIENCE

Pioneering studies in malnourished children have described abnormal EEG patterns, mostly characterized by reduction in amplitude and frequency of the brain waves as compared with the EEGs of control children and usually associated with other clinical alterations (Engel, 1956). In an EEG study on 46 South African malnourished children, Nelson and Dean (1959) found abnormal focal discharges in 36% of the cases, suggesting an enhancement of excitability, as found in certain pathologies such as epilepsy. Such findings could lead to the hypothesis that malnourished humans would present a higher incidence of epilepsy as compared with well-nourished controls. In humans, however, the relationship between nutritional deficiency and seizure susceptibility has not been much investigated in the last three decades. More recently, follow-up studies have shown a higher prevalence of developmental delay and neurological disabilities in low-birth-weight preterm human newborns (see Almeida et al., 2002), and a tendency of malnourished children to develop epilepsy has also been reported (Nunes et al., 1999; Hackett and Iype, 2001). Although suggestive, these studies did not represent the definitive confirmation of the causal relationship between malnutrition and epilepsy in humans. The acceptance of this hypothesis still needs systematic and robust investigations, similar to those already available in laboratory animals.
The earlier EEG studies in humans were essentially based on the visual analysis of the EEG by employing experienced observers trained in doing visual EEG scoring and in recognizing in it certain defined electrographic patterns. Although useful for qualitative analysis, this technique cannot avoid certain subjectivity. Quantification of parameters such as amplitude, frequency, and rhythmicity cannot be satisfactorily achieved with the visual analysis technique (Morgane et al., 1978). The employment of spectral analysis and fast Fourier transform algorithms to quantify EEG features (see Morgane et al., 1978) allowed studying the ontogeny of EEG patterns both in children (Schulte and Bell, 1973) and in laboratory animals. In the latter subjects, several studies have provided important information on EEG patterns under nutritional deficiency, reporting alterations in frequencies and amplitudes of the EEG waves (Salas and Cintra, 1975). A delay in the maturation of the EEG pattern in malnourished rats has also been observed (Gramsbergen, 1976). Also, in the 1970s, Peter J. Morgane and coworkers developed a series of EEG studies in the rat, forming an

FIGURE 4.2 Multunit spike responses (right traces) recorded extracellularly with a multibarrel glass micropipette during periods of iontophoretically ejected pulses of the excitatory amino acid glutamate (10 nA; 30 sec) through the same pipette assembly. Left traces are DC recordings of the slow potential change of spreading depression (SD; see Section 4.4). SD was elicited by topical application of KCl (a cotton ball 1 to 2 mm in diameter soaked in a 2% KCl solution for 1 min) to a point of the cortical surface of an anesthetized adult rat. Recordings 1 and 2 at the right were taken at time points marked by numbers 1 and 2 at the left tracings (respectively before and during SD). (A) Control condition, showing the glutamate-elicited spike activity (A1), which disappeared during SD (A2). (B) Same as in (A), except that during the entire sequence of SD elicitation and DC recording (showed at left) naloxone hydrochloride (50 nA) was ejected iontophoretically while the neuronal responses to glutamate (shown at right) were recorded through the same pipette assembly. Naloxone ejection during SD (tracing B2) prevented the disappearance of neuronal spike activity (compare with tracing A2 taken during SD in the absence of naloxone), suggesting an antagonistic action of naloxone on SD (see also Table 4.1). (From Guedes, R.C.A. et al. (1987b) Experimental Brain Research 39, 113–118. With permission.)
EEG power spectral atlas describing EEG ontogeny (see Morgane et al., 1978). EEG recordings and power spectral plots from several brain regions at different ages and in distinct stages during the sleep–wakefulness cycle provided a good description of the developmental EEG features in the albino rat. By comparing animals fed a control diet (25% protein) with those fed a protein-deficient one (8% protein), it was found that the main EEG disturbances were present in the hippocampus, the protein-deprived rats having more power in the frequency range of 3.5 to 10 Hz (which includes the theta waves) both during REM sleep and waking (Morgane et al., 1978). A recent EEG study of the rat circadian sleep–wake cycle has confirmed the higher EEG power in the theta range of frequencies in the malnourished group, as compared with the controls; the authors were also able to describe significant homeostatic and circadian alternations in the sleep–wake cycle (Cintra et al., 2002). Interestingly, a lower threshold for inducing seizures by the kindling technique was found in the hippocampus of malnourished rats (Bronzino et al., 1986). Other experiments performed with distinct paradigms of nutritional deficiency and different seizure models also indicate lower threshold for seizures in nutritionally deficient rats (Stern et al., 1974; Palencia et al., 1996). In contrast to this idea, two sets of experiments performed by the Morgane group (Morgane et al., 1978) showed that (1) penthylenetetrazole (PTZ)-induced seizures occurred in malnourished rats after latencies comparable to those of well-nourished animals and (2) motor seizures induced by repeated stimulation of the amygdala (kindling model of epilepsy) revealed higher thresholds in malnourished rats as compared with control rats. Taken together, the results are compatible with the idea that the facilitatory effect of malnutrition on seizure susceptibility in the rat depends on the method used to induce seizures and the brain structure involved in each case. Whether this is also the case for the human brain remains to be determined.

4.3 EVOKED POTENTIALS AND NUTRITION

During an EEG recording session, the spontaneous activity pattern can be modified by the appearance of evoked responses consequent to sensory stimulation. At the peripheral level, it is possible to elicit evoked responses by stimulating sensory receptors with the adequate forms of energy. Under such a situation, an evoked response will appear at the functionally corresponding region of the brain. For example, application of light stimuli to the photoreceptors of the retina will elicit evoked potentials in the occipital cortex; on the other hand, such evoked responses will be recorded in the temporal cortical region when one applies a sound stimulus to the auditory receptors of the cochlea at the inner ear.

Electrical current pulses, instead of sensory stimuli, can also be used to produce evoked responses. In this case, electrical stimulation can be applied either at a peripheral afferent structure (such as the sciatic nerve) or centrally (at the brain tissue). These responses can be recorded either peripherally (at another point on the afferent or efferent pathway that is under stimulation) or centrally (by recording the responses at brain level). In summary, when using electrical stimulation, one can activate a peripheral nerve and record either peripherally, at a remote point of the same nerve, or centrally, at the brain level; responses elicited by central (brain) stimulation can also be recorded either centrally, at another brain area of the same or the opposite hemisphere, or peripherally, at an efferent pathway. In all cases, it is necessary that both stimulated and recorded areas be functionally connected.

Brain-evoked potentials may provide valuable clues on how the nervous system transmits and processes information, both at the peripheral and at the central levels. Disturbances in these processes may be revealed by recording and analyzing evoked responses. Thus, it can be very useful to compare evoked potential features in organisms submitted to distinct nutritional conditions in order to detect the effects of such situations on transmitting and processing peripheral sensory information. Increases in the latencies of visually and transcallosal electrically evoked responses have been found at an early age (14 to 20 days) but not later in adult life (95 to 100 days and 60
to 65 days, respectively) in early protein-deprived rats as compared with controls (Morgane et al., 1978). Auditory-evoked potentials (Rocinholi et al., 2001) and electrically evoked action potentials in sensory nerves (Segura et al., 2001) of early malnourished rats are also delayed. In anesthetized and paralyzed chronically malnourished rats, sciatic nerve action potentials provoked by electrical stimulation presented nearly a 50% reduction in the conduction velocities as compared with well-nourished animals (Silva et al., 1987; Figure 4.3). Responses recorded in the corticospinal tract following surface stimulation of the motor cortex were reduced about 15.5% in early malnourished rats as compared with well-nourished, age-matched controls (Quirck et al., 1995). In malnourished children, motor and sensory nerve conductions are shown to be significantly impaired both in peripheral and in central pathways (Chopra, 1991; Tamer et al., 1997). In some cases, histological investigation has indicated a decrease in diameter of myelinated fibers as well as signs of delayed myelination and axonal degeneration. All the clinical data suggest altered functional status, which could be associated with clinical signs such as muscle weakness, hypotonia, and hyporeflexia, usually found in malnourished children. Such signs could be involved in generating learning deficits and impairment of hand dexterity and motor coordination, among other functional deficiencies linked to malnutrition (Chopra, 1991).

4.4 NUTRITION AND CORTICAL SPREADING DEPRESSION (SD)

Cortical SD was first described by Leão (1944) as a reversible and propagated wave of reduction (depression) of the spontaneous and evoked electrical activity of the cerebral cortex, with a simultaneous slow potential change (also called DC potential change) of the tissue, in response to the electrical, chemical, or mechanical stimulation of one point of the cortical surface. The term depression, in this context, does not refer to the psychiatric disease, named also by that term, but rather has an electrophysiological meaning: it signifies that the amplitude of the EEG activity at a certain cortical region becomes temporarily depressed, i.e., the potential difference between two recording points in the depressed area tends to zero. In some cases, the EEG trace actually becomes isoelectric (the amplitudes of the oscillating EEG waves equal to zero). Simultaneously to the EEG depression, the DC potential of that cortical point (measured with a DC amplifier against a remote point having a fixed potential, as, for example, the nasal bones) starts to change, becoming progressively negative. This slow potential change can attain maximum values ranging from –5 to –30 mV after 1 to 2 min of onset and is fully reverted after a few minutes. The always present
negative DC potential can be eventually preceded, and more frequently followed, by positive
deflections, usually of smaller amplitudes than those of the negative one. The depression of EEG
activity (as well as the DC potential changes) spreads concentrically from the stimulated point,
reaching gradually more and more remote cortical regions, while the originally depressed area
starts to recover (Figure 4.4). As a rule, the complete recovery of a depressed region is achieved
after about 5 to 10 min, as evaluated by the restoration of the predepression EEG pattern and DC
level. In contrast to the EEG, the slow potential change accompanying SD has all-or-none charac-
teristics, being very useful to calculate the velocity of propagation of the phenomenon. Surprisingly,
in all vertebrate species so far studied, from fishes to mammals, the velocity of SD propagation
was found to be remarkably low (2 to 5 mm/min; Leão, 1944) when compared with the much
higher conduction velocity of neuronal action potentials, which in mammals is in the range of
meters per second. This peculiar SD velocity has led the authors to postulate a humoral mechanism
of propagation based on the release of one or more chemical factors from the neural cells. According
to this idea, as these compounds diffuse through the extracellular space, they "contaminate" the
neighbor cells, which then become depressed, releasing the SD-eliciting chemical factors, which
contaminate other cells, and so on, giving rise to a genuine autoregenerative propagation maintained
by that positive feedback loop.

During these six decades since SD description, a very extensive body of information on SD
phenomenology has accumulated. However, the clarification of its final mechanisms has not yet
been fully achieved, but putative links with three relevant human pathologies — epilepsy, migraine,
and brain ischemia — deserve some comments. During SD, while the spontaneous activity is
depressed, epileptiform waves, similar to those found in the EEG of epileptic patients, eventually
appear (Leão, 1944; Guedes and Do Carmo, 1980; Figure 4.5). This led to the idea that perhaps
SD and epilepsy mechanisms have some common features. The description of brain vascular
changes during SD, similar to those seen in the classical migraine (migraine with aura), also leads
to the association between the two phenomena in terms of common mechanisms (Hadjikhani et

FIGURE 4.4 Scheme illustrating the cycle of events occurring during an episode of SD. Stimulation of a
point (x) of the normal cortical surface (1) can elicit SD (dark area in 2). Once elicited, SD usually propagates
concentrically to remote regions (dark areas in 3 and 4), while the initially depressed area slowly recovers its
normal pre-SD activity (central light area in 5 and 6). Finally, the whole cortical tissue returns back to the
control predepression situation, as in 1.
al., 2001; Lehmenkühler et al., 1993). Finally, the same logic led some authors to postulate an important role for SD in the physiopathology of ischemia (Takano et al., 1996). In all cases, current discussions often mention the possible involvement of certain ions (Guedes and Do Carmo, 1980; Siesjö and Bengtsson, 1989), free radicals produced in the nervous tissue (El-Bachá et al., 1998; Guedes et al., 1996), or neurotransmitter activity. Different neurotransmitters have been shown to be associated with distinct effects on SD; in some cases, a facilitatory effect has been reported (Guedes et al., 1992) and in others an antagonistic effect has been found (Gorelova et al., 1987; Guedes et al., 1987b, 1988, 2002). An overview of the current knowledge about SD features and research trends can be found in Lehmenkühler et al. (1993) and Gorgi (2001), among others.

As regards malnutrition, electrophysiological studies have documented a facilitatory effect on brain ability of early malnourished rats to propagate SD (De Luca et al., 1977). Data from our laboratory have shown that malnutrition early in life facilitates SD propagation in adulthood, as judged by the SD velocities, higher in the early malnourished animals than in controls (Guedes et al., 1987a). The protein supplementation of a diet deficient in quantity and quality of its protein led to distinct results on SD propagation, depending on the quality of the protein used in the supplementation: by supplementing the diet with a low quality (vegetable) protein, the effects on SD were not reverted. Reversion of SD alterations was achieved only when the protein used to supplement the diet was of high quality (casein; Andrade et al., 1990). Indeed, even short periods of malnutrition during lactation can long-lastingly facilitate SD propagation (Rocha-de-Melo and

FIGURE 4.5 Recording of a typical DC change of spreading depression (A) and of the EEG epileptiform activity immediately preceding it (B) in an anesthetized rabbit submitted to systemic reduction of the extracellular chloride concentration by means of gastric washing during the 4-h period preceding SD recording. The inset at the upper left shows the positions of the cortical electrodes for recordings (A) and (B) as well as the extracortical position of the common reference electrode (R) at the nasal bones. Records B1 to B4 were taken during the periods indicated by 1 to 4 in (A). Epileptiform activity was fully developed at time point 2 and became totally depressed at time point 4. The calibration pulse in (A) (vertical bar at left) corresponds to –10 mV. (From Guedes, R.C.A. and Do Carmo, R.J. (1980) Experimental Brain Research 39, 341–349. With permission.)

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Guedes, 1997). In addition, the SD responses of the brain to substances such as diazepam (Guedes et al., 1992) and glucose (Costa-Cruz and Guedes, 2001) are reduced in early malnourished rats in comparison with the responses in controls.

Besides malnutrition, several other clinically relevant conditions, including nutritional and metabolic variables, associated or not with environmental, hormonal, or pharmacological ones, have been investigated. Such conditions, which are known to affect brain development and functioning, can modify considerably the cortical tissue ability to present and to propagate SD. For example, increased brain susceptibility to SD has been observed in conditions such as (1) systemic reduction of extracellular chloride levels (Guedes and Do Carmo, 1980); (2) deprivation of REM sleep (Amorim et al., 1988); (3) hypoglycemia (Costa-Cruz and Guedes, 2001); (4) systemic increase of the GABAergic activity by diazepam (Guedes et al., 1992); (5) ingestion of ethanol (Guedes and Frade, 1993); (6) hyperthyroidism early in life (Santos, 2000); and (7) dietary deprivation of antioxidant vitamins (El-Bachá et al., 1998; Guedes et al., 1996). In contrast, other experimental situations have been shown to reduce brain susceptibility to SD. These conditions include (1) dietary treatment with lithium (Guedes et al., 1989); (2) hyperglycemia (Costa-Cruz and Guedes, 2001); (3) anesthesia (Guedes and Barreto, 1992); (4) early hypothyroidism (Guedes and Pereira-da-Silva, 1993); (5) aging (Guedes et al., 1996); (6) environmental stimulation (Santos-Monteiro et al., 2000); (7) systemic, topical, and microiontophoretic treatments with the opioid antagonist naloxone (Guedes et al., 1987b); (8) topical cortical application of excitatory amino acid antagonists (Guedes et al., 1988); and (9) pharmacological treatment with drugs that increase brain serotonin activity (Guedes et al., 2002). A summary of studies on the conditions that influence cortical susceptibility to SD and the main effects associated with them are presented in Table 4.1.

SD recording at the cortical surface of the rat brain is performed mostly with the animal under anesthesia, but it can also be performed in the nonanesthetized animal. In this latter case, the previous implantation of the electrodes under anesthesia is necessary; after the electrodes have been fixed on the cortical surface, throughout the skull bones, the animal is allowed to recover from anesthesia and then the recordings can be done in the awake condition (Figure 4.6). By using this technique, we were able to study in rats the effects of two types of anesthesia: (1) that obtained with thionembutal and (2) that produced by a mixture of urethane plus chloralose. In that study, SD features were compared in the same animals during anesthesia and in the awake state (Guedes and Barreto, 1992; see also Table 4.1).

It can be concluded that the electrophysiological recording of the SD phenomenon is a very interesting and valuable tool for studies on both nutrition and brain function and on nutrition and other clinically important conditions that influence the functioning of the nervous system.

4.5 CONCLUDING REMARKS

The generation of electrical activity is the main physiological feature of the nervous tissue. Through this activity, the brain is able to execute the immense repertoire of its actions, from the simplest to the highly complex ones. Therefore, the methods that can be used to record and to analyze the brain electrical activity can provide very important information on the understanding of how it functions under physiological and pathological conditions.

As shown in this chapter, data from electrophysiological investigations, both in laboratory animals and in humans, point to the importance and usefulness of this technique in studying the nervous system under normal conditions and also in understanding how its functioning may be changed by nutritional alterations. For example, attempts to establish the degree of comparability between rat and human sensory evoked potentials, considered as measures of sensory function, have been performed in studies on neurotoxicology in order to examine the extrapolation of data from one species (rat) to the other (humans; Boyes, 1994). It is thereby highly desirable that such techniques become increasingly used in studies in the field of nutritional neuroscience as a
complementary means to understand the relationship among diet, nutrition, and neural development and function.

In our laboratory, we have dedicated much effort and time to this goal by employing the phenomenon of SD as an interesting electrophysiological model. At the beginning (late 1970s), we became very enthusiastic with the publication of a short paper by De Luca et al. (1977) on SD in malnourished rats, because we foresaw the possibility of having, in a short period, a proliferation of research groups interchanging ideas and data about that subject. Unfortunately, with the exception of that pioneer work, to the best of our knowledge no other research groups have decided, till date, to share our efforts on this interface (nutrition and SD) — we consider that a pity. However, we are optimistic about the future of this field of research in view of the recently growing number of

<table>
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<th>Effect</th>
<th>Ref.</th>
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<td>Reduced SD velocities in PTU-treated rats compared with saline-treated controls</td>
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<td>Rat and gerbil</td>
<td>Inverse correlation between age and SD velocity; reduced by dietary antioxidant vitamin deficiency</td>
<td>Guedes et al., 1996</td>
</tr>
<tr>
<td>Early environmental stimulation</td>
<td>Rat</td>
<td>Reduced SD velocities compared with controls</td>
<td>Santos-Monteiro et al., 2000</td>
</tr>
<tr>
<td>Treatment with the opioid antagonist naloxone</td>
<td>Rat and gerbil</td>
<td>Naloxone antagonizes SD incidence and propagation</td>
<td>Guedes et al., 1987b</td>
</tr>
<tr>
<td>Topical cortical application of excitatory amino acid antagonists</td>
<td>Rat</td>
<td>MK-801 antagonizes SD propagation. NMDA and kainic acid facilitate or block it, depending on the dose</td>
<td>Guedes et al., 1988</td>
</tr>
<tr>
<td>Treatment with drugs that increase brain serotonin activity</td>
<td>Rat</td>
<td>Both t-fenfluramine and citalopram antagonize SD propagation</td>
<td>Guedes et al., 2002</td>
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FIGURE 4.6 Representative recordings of the DC slow potential change typical of SD in two rats with implanted Ag-AgCl electrodes (shown in the upper right inset). The animals were anesthetized either with a mixture of urethane plus chloralose (U + C) or with thionembutal (TH). The recording electrodes 1 and 2 were inserted through small holes (about 1 mm in diameter) in the skull until their tips touched the dura mater and were then fixed with dental acrylic. The reference electrode (Re) had its tip implanted inside the nasal bones. Between the reference and the first recording electrodes, a larger hole (2 to 3 mm in diameter) was drilled to expose a portion of the frontal cortical surface and a small plastic ring (Ri) was fixed around it with dental acrylic. Through this hole, the SD-eliciting stimulus (a small cotton ball of 2 to 5% KCl solution for 1 min) was applied during successive recording sessions. In the 24- to 48-h period between two consecutive SD recording sessions, this plastic ring was kept filled with a larger cotton ball (2 to 3 mm in diameter) embedded in mineral oil to prevent air drying of the exposed cortical region. In each animal, an initial recording session was held immediately after electrode implantation, with the animal still under anesthesia (recordings ANEST. I; traces at left column). On the following 3 to 7 days, at least two additional recording sessions were performed in the same animals in the awake state (AWAKE, middle column). Finally, the last session was held 4 to 8 days after electrode surgical implantation. This last session consisted of a 2-h baseline recording, after which the animals were anesthetized again and the recordings continued for an additional 2- to 3-h period (ANEST. F; recordings at right column). The horizontal calibration bar equals 1 min. A –5 mV signal is shown at the beginning of each recording. (From Guedes, R.C.A. and Barreto, J.M. (1992) Brazilian Journal of Medical and Biological Research 25, 393–397. With permission.)

researchers interested in SD, both in the basic and in the clinical area (see Lehmenkühler et al., 1993; Gorgi, 2001).

Two main reasons motivated us to use the SD model: (1) SD provides an interesting and easy way of studying developmental and nutritional aspects of brain electrophysiology, and (2) we strongly believe that the complete understanding of SD mechanisms could be extremely important in helping develop better knowledge and treatment of human neuropathologies such as epilepsy, migraine, and brain ischemia. In this context, and as a final remark, I cite the impressive words of Professor Charles Nicholson on the importance of studying SD mechanisms. In the preface of the book by Lehmenkühler et al. (1993), on the possible role of SD in human migraine, he wrote:
Spreading depression remains seductive — and important. It is important not only because of the compelling evidence that it is the underlying phenomenon of migraine aura but also because it represents a great challenge to the completeness of our knowledge of the brain. No matter how many channel proteins we sequence, how many neuromodulators we identify and how many neural networks we construct, if we cannot explain spreading depression, we do not understand how the brain works.

Exaggerated? Overestimated? Highly biased opinion? I do not think so, but maybe some readers do. For those readers, here is the challenge: get some more information on the SD phenomenon. This can easily be achieved by consulting the pertinent literature cited in this chapter, as well as other seminal works, which can be found with the help of the Internet. Perhaps after that these readers will change their mind. And if they do not — well, they will find, for sure, an excellent way of learning and practicing electrophysiological techniques currently used to study brain development and function.

REFERENCES


