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# Maturation of convulsogenic activity induced by leptazol in the albino rat

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## Summary

1. Maturation of the excitatory and inhibitory neuromechanisms at various levels of the central nervous system was demonstrated by the convulsogenic activity induced by leptazol in the developing albino rat.

2. The somatomotor end points considered (myoclonic jerk, myoclonic seizure, tonic seizure and catalepsy) were not observed in all age groups. Tonic seizure was seen at birth, myoclonic jerks at 2 weeks of age, myoclonic seizure and catalepsy at 3 weeks of age.

3. The convulsive sequences described presented three different patterns, defining three age groups: the infant pattern (infant group: newborn–1 week old animals); the transitional pattern (transitional group: 2 week old animals); and the adult pattern (adult group: 3 week old–adult animals).

4. Effective doses were determined for the three types of convulsive sequence: MJ50 for the myoclonic major sequence (maximal end point: myoclonic jerk), MS50 for the myoclonic major sequence (maximal end point: myoclonic seizure) and the TS50 for the myoclonic-tonic-clonic sequence (maximal end point: tonic seizure).

5. The correlation of the convulsive patterns with the dose and latency variations suggests that: (a) the neuromechanisms responsible for the tonic seizure and clonic seizure, located at brainstem and spinal cord levels, function at birth and reach maturity at 3 weeks of age; (b) the neuromechanisms responsible for the myoclonic manifestations and for catalepsy, located at the striato-thalamo-cortical level, start functioning at 2–3 weeks of age, indicating the later maturation of the more cephalic structures.

## Introduction

The convulsogenic activity induced by leptazol depends on the stimulation of interacting excitatory and inhibitory neuromechanisms, located at various levels of the central nervous system. According to the dose used, neuromechanisms at one or more level can be activated (Gastaut & Fischer-Williams, 1959; Hahn, 1960; Esplin & Zablocka, 1965). The induced nervous discharges are translated into different somatomotor responses, which can be used as an index of the activity of the neural levels stimulated.

Observation of the responses induced by leptazol in a developing animal permits the degree of maturity of the stimulated neural levels to be determined using as a

reference the pattern of the responses and the time of their appearance. Modifications of the responses during development depend on the progressive maturation of the neural levels responsible for the diverse manifestations.

This paper describes the results of such an analysis in the albino rat, in various phases of its postnatal development. In a previous paper (Engelhardt & Esbérard, 1968) we studied the reactivity of the neuromechanisms of the spinal cord and caudal regions of the brainstem using strychnine. This paper demonstrates not only the reactivity of the caudal neuromechanisms, but also that of the more cephalic regions. A brief communication of some of the results has already been published (Engelhardt, Esbérard & Casrilevitz, 1970).

Previous descriptions of the effect of leptazol on the immature animal are restricted to those of Libet, Fazekas & Himwich (1941) and Cadilhac, Passouant-Fontaine, Mihailovic & Passouant (1962), who studied the electroencephalographic modifications induced in the immature cat; Caveness, Nielsen, Adams & Yakovlev (1960), who studied focal seizures in the immature monkey; and Vernadakis & Woodbury (1969), who studied the variation of the median effective dose in the developing rat.

## Methods

Eight hundred and sixty-two albino rats (Wistar strain) of both sexes were distributed into six age groups (Table 1). Leptazol (10–100 mg/ml) was injected intraperitoneally in doses of 25–400 mg/kg. In newborn and 1 week old animals the skin had minimal elasticity and the injections were made through a small strip of plastic adhesive applied to the abdominal wall, to avoid reflux of the injected solution through the needle puncture.

The rats were individually observed after the injection and four to six animals were studied in each session. A set of records was made by one observer for each animal throughout the reactive period. As a result, each age group and each dose was studied by more than one observer and a final set of records was established for each age group and each dose from the individual records obtained.

Each rat was used only once, to avoid responses caused by modifications of reactivity (Liebert & Weil, 1939; Strecker, Alpers, Flaherty & Hugues, 1939; Whitehead, Neubürger, Rutledge & Silcott, 1940; Sacks & Glaser, 1941).

### *End point criteria*

End points were established, following the methods of Finkelman, Steinberg & Liebert (1938), Strauss & Landis (1938), Strauss, Landis & Hunt (1939), Toman,

TABLE 1. *Characteristics of animals used*

| Age group | Age (days) | Number of animals | Number of litters | Weight (g) |
|---|---|---|---|---|
| Newborn | 1–3 | 189 | 35 | 5·4± 1·01 |
| 1 week | 6–8 | 100 | 19 | 9·3± 1·85 |
| 2 weeks | 13–15 | 104 | 26 | 15·6± 3·70 |
| 3 weeks | 20–22 | 155 | 29 | 20·9± 4·56 |
| 4 weeks | 27–29 | 137 | 26 | 32·6± 5·03 |
| 8 weeks | 54–58 | 93 | 24 | 95·7±18·66 |
| Adult | 180–360 | 84 | — | 197·9±33·87 |
| Total | | 862 | | |

Age in days refers to range in each age group. Weight values are given as mean ±S.D.

Swinyard & Goodman (1946), Radouco, Frommel, Gold, Greder, Melkonian, Radouco, Strassberger, Vallette & Ducommun (1952), Woodbury & Davenport (1952), Jenney & Pfeiffer (1956), Truitt, Ebersberger & Ling (1960), and Esplin & Zablocka (1965). These authors used electroshock stimulation, leptazol and hexafluorodiethyl ether to produce convulsions in various species.

The end points observed on mature animals in this paper are as follows: (1) Myoclonic jerk (MJ), consisting of a sudden episode of head extension-retraction, with abduction-protraction of the hind limbs and clonic movements of one of the fore limbs, followed by a return to normal posture ; duration 1–2 seconds. (2) Myoclonic seizure (MS), a sustained episode of head retraction-flexion-torsion, followed by abduction-protraction of the hind limbs, with possible elevation of the head and trunk or partial or total loss of quadruped posture and clonic movements of the limbs, followed by a return to normal posture ; duration 10–60 seconds. (3) Tonic seizure (TS), comprising (a) anterior tonic seizure (aTS), characterized by loss of quadruped posture, head hyperextension, extension of trunk-pelvis-tail, caudal extension of the fore limbs and flexion of the hind limbs—sustained for 5–30 s ; and (b) complete tonic seizure (cTS), characterized by additional caudal extension of the hind limbs—maintained for 15–30 seconds. (4) Catalepsy, the animal remains still, presenting great tolerance to manipulation, sustaining abnormal postures without reaction, with delayed recovery.

In immature age groups, end points may be absent or they may appear with different features. Differences are emphasized in **Results**. Other features, less clear-cut, were not used as end points, but are described for sake of continuity.

### *Quantitative determinations*

Median effective doses were calculated according to Finney (1952, 1962) for myoclonic jerk (MJ50), myoclonic seizure (MS50), anterior tonic seizure (aTS50) and complete tonic seizure (cTS50).

The mean latent period for the first myoclonic jerk, the first myoclonic seizure and the first anterior tonic seizure of a given convulsive sequence corresponds to the mean value of the delay observed in the reaction of animals of each age group.

## Results

### *Patterns of somatomotor responses*

The chief features of the somatomotor responses observed in the various age groups, in the order of their appearance, are described in Table 2. The correlation of the response modalities observed in the several age groups examined permitted the separation of three patterns of response, which characterize three age groups: (1) the infant pattern, observed in the infant group (newborn and 1 week old animals) ; (2) the transitional pattern, corresponding to the transitional group (2 week old animals) and (3) the adult pattern, characteristic of the adult group (animals 3 weeks old or more).

According to the dose related to the age group, three types of convulsive sequence were observed. Each sequence was also related to the three patterns of response. (1) Myoclonic minor sequence was observed with doses corresponding to the MJ50. This sequence was well defined in the adult age group, starting with hyperkinesia

TABLE 2. *Sequences of responses to leptazol*

| | Adult group (3 weeks old to adult) | Transitional group (2 weeks old) | Infant group (newborn and 1 week old) |
|---|---|---|---|
| Hyperkinesia | Slightly increased activity, short runs, intense whisker activity. | Greatly increased activity, progression and regression, less intense whisker activity. | Very intense activity, progression and regression, episodes of loss of quadruped posture. |
| Myoclonic jerk | Quick episode (1–2 s) of head extension-retraction with postural reinforcement of hind limbs and clonic jerks of fore limbs. Up to thirty or more, isolated or in volleys. Constant. | Slow episode of head extension and abduction of the four limbs. Few and usually isolated. With less frequency, similar to those of the adult. Not constant. | Not observed. Continuation of hyperkinesia with increasing activity and frequent episodes of loss of quadruped posture and slow movements of head–trunk–pelvis and limbs (contortions) and delayed righting. |
| Myoclonic seizure | Episode (10–60 s) of head retraction–flexion–torsion with no loss, partial or total loss of quadruped posture and clonic jerks of the limbs. One, two, rarely more. Constant. | Atypical episode of head extension–retraction, loss of quadruped posture and uncoordinated limb movements associated with ill defined clonic movements. Rare. | Not observed. Hyperkinesia as described above continues. |
| Tonic seizure | Preceded by fast run, sudden stop, extension of head–trunk–pelvis, loss of quadruped posture; fore limbs are slowly extended caudalwards and hind limbs flexed constituting the aTS posture. aTS is sustained for 5–10 s and followed by caudalwards slow extension of hind limbs to constitute cTS, or it is sustained for 15–30 s and followed by clonic seizure. Rarely repeated in the same reactive period. cTS is infrequent in 8 week old and adult rats, and very frequent in those of 3 and 4 weeks. Constant. | Quick progression, sometimes with adult characteristics, loss of quadruped posture and establishment of aTS posture. cTS is very frequent. Tonic postures present features similar to those of the adult, being more prolonged (up to 45 s). Tonic seizure, anterior or complete usually is repeated several times (up to eight) in the same episode. Constant. | Progression followed by loss of quadruped posture, slow contortions and establishment of aTS posture. cTS is very frequent. Tonic postures have characteristics similar to those of the adult, being maintained longer (usually from 30 to 60 s, reaching 120 s). Tonic seizure is repeated several times (ten or more) in the same reactive period. Constant. |
| Clonic seizure | Follows aTS or cTS immediately. Initially very intense with generalized clonic jerks and extensor spasms of the hind limbs. Proceeds with gradual decrease of intensity. Lasts 5–15 min or more. | Follows tonic seizure, immediately or after a short period of depression. Less intense than in the adult group, clonic jerks are poorly characterized and extensor spasms of hind limbs are absent. Lasts 15 min or more. | Observed after a prolonged period of depression (30–120 s) that follows tonic seizure. Consists of slow contortions and tremors of the limbs, gradual decrease of intensity. Duration of 30 min or more. |
| Catalepsy | The rat remains curled up, does not react to manipulation nor to the imposition of abnormal postures. More frequent, intense and prolonged after the myoclonic seizure, also observed after the myoclonic jerk or the clonic seizure. Duration 5–30 min or more. | Not observed. | Not observed. |

followed by myoclonic jerks. In the transitional age group, myoclonic jerks could be absent or atypical. In the infant age group only hyperkinesia was observed. The reactive period, which started 1–2 min after the injection, lasted for 5–30 minutes. (2) Myoclonic major sequence was observed with doses corresponding to the MS50. In the adult group it was well defined, comprising hyperkinesia, myoclonic jerks and myoclonic seizure. In the transitional group it was usually incomplete, lacking the myoclonic seizure. This sequence was absent in the infant group, in which only hyperkinesia could be observed for this dose. The duration of the reactive period varied between 10 and 45 min, but it could last, less frequently, up to 90 minutes. (3) Myoclonic-tonic-clonic sequence was observed with doses corresponding to the TS50. It was well defined in the adult group, in which it comprised hyperkinesia, myoclonic jerks, myoclonic seizures and the tonic-clonic period. The tonic-clonic period started with tonic seizure and covered all the events until the return to quadruped posture. This sequence was frequently incomplete in the transitional group, where the myoclonic manifestations could be absent. In the infant group it consisted of hyperkinesia followed by the tonic-clonic episode. The reactive period, starting 30–60 s after injection, in the transitional and adult groups usually lasted for 30–90 min, sometimes reaching 120 min ; in the infant group it varied from 90 to 120 min but could extend to 240 minutes.

### *Variation of the median effective doses in relation to age*

Three median effective doses were determined (Table 3): (1) MJ50, required to evoke the myoclonic minor sequence, occurring from the transitional group onwards, with only small fluctuations ; (2) MS50, required to evoke the myoclonic major sequence, occurring only in the adult group, the variations of which were not significant ; (3) TS50, required to evoke the myoclonic-tonic-clonic sequence, occurring in all age groups. Two doses were calculated: one producing anterior tonic seizure (aTS50) ; the other, somewhat higher, producing complete tonic seizure (cTS50). The median effective dose for anterior tonic seizure, a response constant and common to all age groups, decreased from birth up to 2–3 weeks of age, and increased from this point onwards. The appearance of hind limb tonic extension characteriz-

TABLE 3. *Median effective doses (mg/kg, i.p.)*

| Age group | Anterior tonic seizure (aTS) | Complete tonic seizure (cTS) | Myoclonic jerk (MJ) | Myoclonic seizure (MS) |
|---|---|---|---|---|
| Newborn | 207·1 (188·4–227·7) | 313·6 (222·5–441·9) | — | — |
| 1 week | 103·4 (82·1–102·7) | 122·4 (38·4–390·3) | — | — |
| 2 weeks | 63·0 (57·0–69·6) | 102·7 (81·6–129·4) | 41·0 (38·8–43·3) | — |
| 3 weeks | 61·3 (53·6–70·2) | 85·2 (72·1–100·7) | 39·0 (37·4–40·7) | 63·8 (56·3–72·4) |
| 4 weeks | 81·5 (73·1–90·8) | 131·5 (126·9–136·5) | 52·0 (47·9–56·4) | 59·6 (55·6–63·8) |
| 8 weeks | 76·1 (69·6–83·3) | 144·1 (74·6–278·5) | 48·1 (38·4–60·2) | 60·0 (43·5–82·0) |
| Adult | 80·0 (31·5–200·0) | — | 32·0 (27·5–37·3) | 52·8 (46·1–60·5) |

Values in brackets are the fiducial limits for chance sampling variation ($P=0·05$).

ing complete tonic seizure, was variable in older animals, as reflected in the wider range of the higher median effective dose required to evoke this sign.

Comparison of the median effective doses for anterior tonic seizure in different age groups showed that the adult value appears initially between 1 and 2 weeks of age, marking the time when the rat shows maximum reaction to the adult dose (Table 3).

*Variation of the mean convulsive latent period in relation to age*

The mean latency was calculated as an index of the modification of the animal's reactivity to the convulsogenic drug in the course of development. It was realized, however, that this was a somewhat artificial value, since there was latency variation for each dose ; higher doses showed the smaller latency. The values of the mean convulsive latent period for the end points considered in the present paper are listed in Table 4.

The latency for anterior tonic seizure had a higher value in the newborn, but from 1 week of age variations in latency were not significant. The variation in latencies for myoclonic jerk and myoclonic seizure was also of no significance.

## Discussion

The somatomotor convulsive reactions to leptazol have been grouped in three sequences, depending on the dose used: the myoclonic minor sequence, the myoclonic major sequence and the myoclonic-tonic-clonic sequence. Three patterns of response were recognized, referring to three age-groups: the infant pattern, the transitional pattern and the adult pattern (Table 2).

The sequences in the immature and mature animal are similar to those reported with bemegride (Vulpe, Caldwell, Rodin, 1963) and with hexafluorodiethyl ether (Webb & Davis, 1964). In the first paper two sequences were distinguished, one having its end point at the myoclonic seizure and another with its end point at the tonic seizure ; in the second paper the sequences described are the maximal ones, since the inhalation of gas was interrupted only after obtaining the tonic seizure. Using electroshock stimulation it has also been possible, according to the intensity of the stimulus, to obtain comparable aspects of the convulsive reactions (Millichap, 1957 ; Vernadakis & Woodbury, 1965, 1969), the maximal end points also differing in the various age groups.

The times of appearance of the end points considered here (myoclonic jerk, myoclonic seizure, anterior tonic seizure) generally agree with those described by

TABLE 4. *Convulsive latent periods*

| Age group | Anterior tonic seizure (aTS) | Myoclonic jerk (MJ) | Myoclonic seizure (MS) |
|---|---|---|---|
| Newborn | 6·35±2·04 | — | — |
| 1 week | 4·11±2·00 | — | — |
| 2 weeks | 3·23±2·43 | 1·92±1·15 | — |
| 3 weeks | 4·12±3·12 | 1·98±1·70 | 2·27±1·54 |
| 4 weeks | 3·67±1·53 | 1·70±0·90 | 2·27±1·41 |
| 8 weeks | 3·45±2·40 | 1·42±0·52 | 1·62±0·62 |
| Adult | 3·67±2·19 | 1·50±0·82 | 1·69±1·40 |

Time values are given as mean±S.D., expressed in minutes.

Vulpe *et al.* (1963), for bemegride, and by Webb & Davis (1964) for hexafluorodiethyl ether. They differ from the findings of Millichap (1957) for electroshock stimulation and from those of Vernadakis & Woodbury (1965, 1969) for leptazol and electroshock stimulation.

Most studies of convulsions on the developing animal suggest that younger animals are less sensitive, sensitivity increasing with maturation. This has been shown for chemical stimulants like strychnine (Falck, 1884, 1885 ; Engelhardt & Esbérard, 1968), leptazol (Libet *et al.* 1941 ; Caveness *et al.*, 1960), bemegride (Vulpe *et al.*, 1963) and hexafluorodiethyl ether (Webb & Davis, 1964). The last two substances induce convulsive manifestations similar to those obtained with leptazol, and they seem to act upon the central nervous system through a common mechanism (Rodin, Rutledge & Calhoun, 1958 ; Chatrian & Petersen, 1960 ; this paper, Table 3). With electroshock stimulation also a more intense current is required to obtain a given convulsive end point in the immature animal (Millichap, 1957 ; Vernadakis & Woodbury, 1965).

These findings, with which our results agree, show that the immature animal is less sensitive to convulsogenic substances such as leptazol, and others that have a similar mode and site of action.

Vernadakis & Woodbury (1969) have also reported on the variation of the median effective dose of leptazol in the developing rat. They used as references the maximal end points obtained by electroshock stimulation (also described by Millichap, 1957), that are different in each age group. Tonic seizure, for instance, was observed only from 2 weeks of age onwards, although other authors, including ourselves, who used chemical stimulants of comparable action (see above) have observed this reaction in the newborn. It seems that Vernadakis & Woodbury (1969) used different end point criteria for the first 2 weeks of age. It is possible, therefore, that the variation they report for the dose effective at each age group results from their failure to discriminate different end points.

### *Maturation of integrative levels*

All levels of the central nervous system are stimulated by leptazol (Schoen, 1926 ; Gutierrez-Noriega, 1944 ; Hahn, 1960 ; Esplin & Zablocka, 1965), including excitatory and inhibitory neuromechanisms (Lewin & Esplin, 1961 ; Esplin & Zablocka-Esplin, 1969 ; Jinnai, Mogami, Mukawa, Iwata & Kobayashi, 1969). The somatomotor convulsive manifestations originate from a process of spatial summation of impulses in different regions of the central nervous system. These become effective when they reach the spinal and supraspinal motor centres—responsible for the somatomotor reactions, where they reinforce local excitation (Gutierrez-Noriega, 1943, 1944 ; Hahn, 1960). The convulsive impulses probably originate in specific regions of the central nervous system, which are important for the initiation and integration of the various aspects of the convulsive sequence (Hahn, 1960).

Correlation of the convulsive patterns with the median doses necessary to produce them and the latency of the appearance of the chief end points (Tables 2–4), allows an assessment of the convulsogenic maturity of the several stimulated levels.

### *Tonic seizure and clonic seizure*

The tonic seizure represents the maximal manifestation of leptazol stimulation of excitatory neuromechanisms that are located, for the most part, at the brainstem

level. The reticular formation is a neuronal structure situated here which, when stimulated, permits the initiation and the development of the self-sustained convulsive attack, without the necessity for significant intervention of circuits rostral to the mesencephalon (Schoen, 1926 ; Asuad, 1940 ; Gutierrez-Noriega, 1944 ; Freedman & Moossy, 1953 ; Starzl, Niemer & Forgrave, 1953 ; Jolly & Steinhaus, 1956 ; Kreindler, Zukerman, Steriade & Chimion, 1958 ; Gastaut & Fischer-Williams, 1959 ; Bircher, Kanai & Wang, 1962 ; Bergman, Costin & Gutman, 1963 ; Kreindler, 1965 ; Sille & Sayers, 1967 ; Jinnai *et al.*, 1969). The reticular formation acts on the effector neurones, particularly on those of the spinal cord, through several descending pathways that transmit the convulsive impulse (Ajmone-Marsan & Marossero, 1950 ; Gastaut & Fischer-Williams, 1959).

The spinal reflex system has an important role in the integration of the maximal convulsive discharges, in the form of stereotyped patterns. These are due to peripheral and supraspinal influences, associated with a background state proper to the spinal cord (Ten Cate, 1950 ; Esplin & Laffan, 1957 ; Woodbury & Esplin, 1959 ; Esplin & Freston, 1960). Leptazol acts on the spinal cord, producing a clear excitatory effect, though it is possible to obtain with high doses in spinal animals a tonic seizure, sometimes of short duration, followed by a clonic phase (Schoen, 1926 ; Asuad, 1940 ; Gutierrez-Noriega, 1944 ; Jones & Lombroso, 1955 ; Esplin, 1957 ; Esplin & Curto, 1957 ; Lewin & Esplin, 1961 ; Esplin & Zablocka, 1965 ; Esplin & Zablocka-Esplin, 1969). Therefore, to obtain a well defined tonic seizure, the region of the central nervous system constituting the brainstem and the spinal cord is necessary, and the excitatory neuromechanisms of these structures must be stimulated. To induce the tonic extension of the hind limbs (complete tonic seizure) the dose of leptazol required is higher than that required to obtain the anterior tonic seizure, probably to overcome the resistance of the spinal inhibitory system (Esplin & Zablocka-Esplin, 1969).

The interruption of the tonic seizure and its substitution by the clonic seizure depends on a process of neuronal exhaustion associated with the activity of the inhibitory neuromechanisms that begin to dominate the excitatory ones (Cobb, 1924 ; Vianna Dias, 1954 ; Gastaut & Fischer-Williams, 1959 ; Jung & Hassler, 1960 ; Jinnai *et al.*, 1969). Clonic manifestations in the mesencephalic and even in the spinal animal (Schoen, 1926 ; Asuad, 1940 ; Gutierrez-Noriega, 1944 ; Jones & Lombroso, 1955 ; Gastaut & Fischer-Williams, 1959) suggest the presence of inhibitory mechanisms in these structures, capable of interrupting the tonic seizure and of maintaining the clonic seizure (Lewin & Esplin, 1961 ; Jinnai *et al.*, 1969).

The rat shows at birth tonic seizures that have qualitative features similar to those of the adult. The subsequent clonic seizure is slow and ill-defined in the infant group, reaching the definitive pattern at 3 weeks of age. These facts suggest that the neural levels necessary for the integration of the tonic seizure, such as the excitatory mechanisms of the brainstem and of the spinal cord are in a condition to be stimulated at birth, though they are less sensitive ; sensitivity increases progressively, reaching adult levels between 1 and 2 weeks of age.

The subsequent clonic seizure which represents the interruption of the tonic seizure and to which inhibitory neuromechanisms contribute can also be seen at birth, though with features different from those of the adult. This suggests that the inhibitory mechanisms of the same neural levels are already present in the newborn,

but it is at 3 weeks of age that the seizure acquires the definitive pattern, integrated by mature neuromechanisms.

In the infant group and in the transitional group, there is a clear predominance of the excitatory neuromechanisms, a fact that is characterized by the great duration and repetition of the tonic seizures in the same convulsive sequence and by the rapid decrease in the values of the median effective doses and of the mean latencies. From 3 weeks of age onwards there is a decrease in the duration and repetition of the tonic seizures in the same convulsive sequence and the clonic seizure is well defined, suggesting the maturity of the inhibitory neuromechanisms.

The existence of neuromechanisms at the level of the brainstem and spinal cord that are capable of being stimulated at birth, is also suggested by the study of the maturation of somatomotor responses induced by strychnine (Engelhardt & Esbérard, 1968). The tonic seizure due to this drug results from the liberation of excitatory neuromechanisms from the block exerted by inhibitory neuromechanisms, and it can be observed at birth. The adult sensitivity is also attained between 1 and 2 weeks of age, and the additional decrease of the median effective doses by the age of 3 weeks and its gradual increase from this moment on is parallel to that observed in relation to leptazol.

Thus, with two drugs of different action, a similar pattern is obtained of the maturation of the caudal excitatory and inhibitory neuromechanisms.

### *Myoclonic manifestations*

These responses, preceding the tonic-clonic episode, are the myoclonic jerk and the myoclonic seizure. The myoclonic jerk is a result of the stimulation of excitatory neuromechanisms located at the striato-thalamo-cortical level, inducing a fast episodic excitatory state that is not seen in absence of these structures. The convulsive discharge is directed to caudal levels, releasing the facilitatory reticulo-spinal system, which is responsible for the momentary tonic reinforcement that is characteristic of this neuromuscular manifestation (Schoen, 1926 ; Asuad, 1940 ; Gutierrez-Noriega, 1944 ; Ajmone-Marsan & Marossero, 1950 ; Pollock & Gyarfas, 1952 ; Starzl, *et al.*, 1953 ; Preston, 1955 ; Jolly & Steinhaus, 1956 ; Gastaut & Fischer-Williams, 1959 ; Woodbury & Esplin, 1959 ; Okuma, 1960 ; Bircher, *et al.*, 1962 ; Esplin & Zablocka, 1965).

The presence of interacting inhibitory neuromechanisms, the thalamo-caudate inhibitory system, capable of actively inhibiting the thalamic and brainstem reticular formations, is suggested by the short duration, the abrupt ending and the frequency of the repetitions of the myoclonic jerks (Gastaut & Fischer-Williams, 1959 ; Jung & Hassler, 1960 ; Lewin & Esplin, 1961 ; Esplin & Zablocka-Esplin, 1969 ; Jinnai *et al.*, 1969).

The myoclonic seizure is seen after a series of myoclonic jerks at diminishing intervals, and seems to be the result of their fusion. The higher median effective dose (MS50) that induces this reaction may correspond to the additional dose necessary to stimulate the excitatory neuromechanisms, intensively enough to prevent the influence of the inhibitory neuromechanisms, thus permitting a sustained excitatory state, responsible for the myoclonic seizure.

The myoclonic manifestations can be observed on the developing rat at 2 weeks of age, although often atypical, and it is from 3 weeks of age onwards that they

appear with adult characteristics. The absence of significant variations of these median effective doses and of the mean latencies with age indicate that there is practically no modification of the reactivity of the neuromechanisms responsible for these manifestations, during development. The myoclonic manifestations therefore attain maturity at the same time, suggesting their integration by a common neuromechanism.

### *Catalepsy*

This manifestation encompasses hypokinetic phenomena of the experimental catatonic syndrome and comprises the loss of motor initiative and the maintenance of abnormal postures (DeJong & Baruk, 1930 ; DeJong, 1956 ; Beaulnes & Viens, 1961). Catalepsy is the result of inhibitory activity at cortical and striatal levels, and it can be elicited by leptazol and other agents (Ingram, Barris & Ranson, 1936 ; Dille & Hazleton, 1939 ; DeJong, 1945 ; Ottaviano & Pappalardo, 1946 ; Baruk, 1950 ; Gutierrez-Noriega, 1951 ; Pollock & Gyarfas, 1952 ; Radouco *et al.*, 1952 ; Konradi, 1953 ; Starzl, *et al.*, 1953 ; Mattioli-Foggia & Cossio, 1957 ; Zetler & Moog, 1958 ; Gastaut & Fischer-Williams, 1959 ; Jung & Hassler, 1960 ; Pavlov, 1961 ; Ashford, Sharpe & Stephens, 1963 ; Steg, 1964 ; Munkvad, Pakkenberg & Randrup, 1969). In the rat it can be observed from 3 weeks of age onwards, that is, only in the adult group, representing the maturity of the inhibitory activity of the striato-cortical level.

From the discussion above we may conclude that the excitatory and inhibitory neuromechanisms responsible for the integration of the tonic seizure, and for the clonic seizure, located in the brainstem and at spinal cord levels, show functional capacity at birth, although with lesser sensitivity, and they reach maturity at 3 weeks of age. The excitatory and inhibitory neuromechanisms responsible for the myoclonic manifestations and for catalepsy, located at striato-thalamo-cortical level, respond to leptazol stimulation at 2–3 weeks of age, indicating the later maturation of these more cephalic structures.

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## REFERENCES

AJMONE-MARSAN, C. & MAROSSERO, F. (1950). Electrocorticographic and electrochordographic study of the convulsions induced by cardiazol. *Electroenceph. clin. Neurophysiol.*, **2,** 133–142.

ASHFORD, A., SHARPE, C. J. & STEPHENS, F. F. (1963). Thymol basic ethers and related compounds: central nervous system depressant action. *Nature, Lond.*, **197,** 969–971.

ASUAD, J. (1940). Contribution a l'étude de l'épilepsie expérimentale chez les animaux décérébrés mésencéphaliques, protubérantieles, bulbaires et spinaux. *Presse méd.*, **48,** 1043–1047.

BARUK, H. (1950). *Précis de Psychiatrie.* Paris: Masson & Cie.

BEAULNES, A. & VIENS, G. (1961). Catatonie et catalepsie. *Rev. can. Biol.*, **20,** 215–220.

BERGMANN, F., COSTIN, A. & GUTMAN, J. (1963). A low threshold convulsive area in the rabbit's mesencephalon. *Electroenceph. clin. Neurophysiol.*, **15,** 683–690.

BIRCHER, R. P., KANAI, T. & WANG, S. C. (1962). Intravenous, cortical and intraventricular dose-effect relationship of pentylenetetrazol, picrotoxin and deslanoside in dogs. *Electroenceph. clin. Neurophysiol.*, **14,** 256–267.

CADILHAC, J., PASSOUANT-FONTAINE, TH., MIHAILOVIC, L. & PASSOUANT, P (1962). Experimental epilepsy in kittens in relation to age: cortical and subcortical study. *Year Book of Neurology, Psychiatry & Neurosurgery*, pp. 167–168. Chicago : The Year Book Publ.

CAVENESS, W. F., NIELSEN, K. C., ADAMS, R. D. & YAKOVLEV, P. I. (1960). Ontogeny of induced seizures in Macaca mulatta. *Trans. Am. neurol. Ass.*, **85,** 90–95.

CHATRIAN, G. E. & PETERSEN, M. C. (1960). The convulsive patterns provoked by indoklon, metrazol and electroshock: some depth electrographic observations in human patients. *Electroenceph., clin. Neurophysiol.*, **12,** 715–725.

COBB, S. (1924). Electromyographic studies of experimental convulsions. *Brain*, **47**, 70–75.

DEJONG, H. H. (1945). *Experimental Catatonia*. Baltimore: The Williams & Wilkins Co.

DEJONG, H. H. (1956). Experimental catatonia in animals and induced catatonic stupor in man. *Dis. nerv. Syst.*, **17**, 135–139.

DEJONG, H. & BARUK, H. (1930). (Eds.) *La Catatonie Expérimentale par la Bulbocapnine*. Paris: Masson & Cie.

DILLE, J. M. & HAZLETON, L. W. (1939). The depressant action of picrotoxin and metrazol. *J. Pharmac. exp. Ther.*, **67**, 276–289.

ENGELHARDT, E. & ESBÉRARD, C. A. (1968). Maturation of somatomotor responses to strychnine in the albino rat. *Br. J. Pharmac.*, **34**, 239–247.

ENGELHARDT, E., ESBÉRARD, C. A. & CASRILEVITZ, MIRA DE (1970). Manifestações convulsivas induzidas pelo cardiazol no rato em desenvolvimento. *Resumos (XXII Reunião Anual S.B.P.C.)*, p. 352.

ESPLIN, D. W. (1957). Effects of diphenylhydantoin on synaptic transmission in cat spinal cord and stellate ganglion. *J. Pharmac. exp. Ther.*, **120**, 301–323.

ESPLIN, D. W. & CURTO, E. M. (1957). Effects of trimethadione on synaptic transmission in the spinal cord; antagonism of trimethadione and pentylenetetrazol. *J. Pharmac. exp. Ther.*, **121**, 457–467.

ESPLIN, D. W. & FRESTON, J. W. (1960). Physiological and pharmacological analysis of spinal cord convulsions. *J. Pharmac. exp. Ther.*, **130**, 68–80.

ESPLIN, D. W. & LAFFAN, R. J. (1957). Determinants of flexor and extensor components of maximal seizures in cats. *Arch. int. Pharmacodyn.*, **113**, 189–202.

ESPLIN, D. W. & ZABLOCKA, B. (1965). Central nervous system stimulants. In: *The Pharmacological Basis of Therapeutics*, 3rd Edit., ed. Goodman, L. S. & Gilman, A., pp. 345–353. New York: The Macmillan Co.

ESPLIN, D. W. & ZABLOCKA-ESPLIN, B. (1969). Mechanisms of action of convulsants. In: *Basic Mechanisms of the Epilepsies*, ed. Jasper, H. H., Ward, Jun. A. A. & Pope, A., pp. 167–183. Boston: Little, Brown and Co.

FALCK, F. A. (1884). Ueber den Einfluss des Alters auf die Wirkung des Strychnins, I. *Pflügers Arch. ges. Physiol.*, **34**, 530–575.

FALCK, F. A. (1885). Ueber den Einfluss des Alters auf die Wirkung des Strychnins, II. *Pflügers Arch. ges. Physiol.*, **36**, 285–308.

FINKELMAN, I., STEINBERG, D. L. & LIEBERT, E. (1938). The treatment of schizophrenia with metrazol by the production of convulsions. *J. Am. med. Ass.*, **110**, 706–709.

FINNEY, D. J. (1952). *Statistical Method in Biological Assay*. London: Charles Griffin & Co. Ltd.

FINNEY, D. J. (1962). *Probit Analysis*, 2nd Edit. London: Cambridge University Press.

FREEDMAN, D. A. & MOOSSY, J. (1953). Effect of mesencephalic lesions on the cortical electro-convulsant threshold. *Neurology (Minneap.)*, **3**, 714–720.

GASTAUT, H. & FISCHER-WILLIAMS, M. (1959). The physiopathology of epileptic seizures. In: *Handbook of Physiology*, section 1: Neurophysiology, vol. 1, ed. Field, J., Magoun, H. W. & Hall, V. E., pp. 329–363. Washington: American Physiological Society.

GUTIERREZ-NORIEGA, C. (1943). Action of metrazol on the motor and sensory nuclei of the brain stem. *J. Neuropath. exp. Neurol.*, **2**, 132–139.

GUTIERREZ-NORIEGA, C. (1944). Interpretacion fisiologica de la acción convulsivante del cardiazol. *Rev. Neuro-psiquiat. (Lima)*, **7**, 14–38.

GUTIERREZ-NORIEGA, C. (1951). La catatonia experimental. *Rev. Neuro-psiquiat., Lima*, **14**, 339–348.

HAHN, F. (1960). Analeptics. *Pharmacol. Rev.*, **12**, 447–530.

INGRAM, W. R., BARRIS, R. W. & RANSON, S. W. (1936). Catalepsy. An experimental study. *Arch. Neurol. Psychiat., Chicago*, **35**, 1175–1197.

JENNEY, E. H. & PFEIFER, C. C. (1956). The predictable value of anticonvulsant indices. *Ann. N. Y. Acad. Sci.*, **64**, 679–689.

JINNAI, D., MOGAMI, H., MUKAWA, J., IWATA, Y. & KOBAYASHI, K. (1969). Effect of brain-stem lesions on metrazol-induced seizures in cats. *Electroenceph. clin. Neurophysiol.*, **27**, 404–411.

JOLLY, E. R. & STEINHAUS, J. E. (1956). The effect of drugs injected into limited portions of the cerebral circulation. *J. Pharmac. exp. Ther.*, **116**, 273–281.

JONES, D. P. & LOMBROSO, C. T. (1955). Effect of metrazol and nembutal on motor activity in the spinal cat. *Am. J. Physiol.*, **180**, 209–214.

JUNG, R. & HASSLER, R. (1960). The extrapyramidal motor system. In: *Handbook of Physiology*, section 1: Neurophysiology, vol. **2**, ed. Field, J., Magoun, H. W. & Hall, V. E., pp. 863–927. Washington: American Physiological Society.

KONRADI, G. (1953). Activity of the nervous system. In: *Text-book of Physiology*, ed. Bykov, K. M. Moscow: Peace Publishers.

KREINDLER, A. (1965). *Experimental Epilepsy. Progress in Brain Research*, vol. **19**. Amsterdam: Elsevier Publishing Co.

KREINDLER, A., ZUCKERMANN, E., STERIADE, M. & CHIMION, D. (1958). Electroclinical features of convulsions induced by stimulation of brain stem. *J. Neurophysiol.*, **21**, 430–436.

LEWIN, J. & ESPLIN, D. W. (1961). Analysis of the spinal excitatory action of pentylenetetrazol. *J. Pharmac. exp. Ther.*, **132**, 245–250.

LIBET, B., FAZEKAS, J. F. & HIMWICH, H. E. (1941). The electrical response of the kitten and adult cat brain to cerebral anemia and analeptics. *Am. J. Physiol.*, **132**, 232–238.

LIEBERT, E. & WEIL, A. (1939). Histopathologic changes in the brain following experimental injections of metrazol. *Arch. Neurol. Psychiat., Chicago*, **42**, 690–699.

MATTIOLI-FOGGIA, C. & COSSIO, M. (1957). Considerazioni neurofisiopatologiche sulla catatonia. *Riv. Patol. nerv. ment.*, **78**, 867–874.

MILLICHAP, J. G. (1957). Development of seizure patterns in newborn animals. Significance of brain carbonic anydrase. *Proc. Soc. exp. Biol. Med.*, **96**, 125–129.

MUNKVAD, I., PAKKENBERG, H. & RANDRUP, A. (1969). *Excerpta med.*, Section 2: Physiology, 22. Abstr. no. 732.

OKUMA, T. (1960). Effect of metrazol on cortical and subcortical evoked potentials. *Electroenceph. clin. Neurophysiol.*, **12**, 685–694.

OTTAVIANO, G. & PAPPALARDO, P. (1946). Ricerche farmacologiche sulla bulbocapnina. (I) Sintomatologia catatonico-extrapiramidale nella tossicosi sperimentale cronica da bulbocapnina. *Boll. Soc. ital. Biol. sper.*, **22**, 1082–1083.

PAVLOV, I. (1961). *La Psychopathologie et la Psychiatrie.* Moscou: Editions en Langues Etrangers.

POLLOCK, G. H. & GYARFAS, K. (1952). Subcortical action of metrazol, tris-B-chlorethyl amine and cyanide. Role of carbon dioxide. *Arch. int. Pharmacodyn.*, **89**, 252–257.

PRESTON, J. B. (1955). Pentylenetetrazole and thiosemicarbazide: a study of convulsant activity in the isolated cerebral cortex preparation. *J. Pharmac. exp. Ther.*, **115**, 28–38.

RADOUCO, C., FROMMEL, E., GOLD, P., GREDER, G., MELKONIAN, D., RADOUCO, S., STRASSBERGER, L., VALLETTE, F. & DUCOMMUN, M. (1952). Etude physio-pathologique de l'action du pentaméthylènetétrazol (cardiazol). *Archs. int. Pharmacodyn. Ther.*, **92**, 13–38.

RODIN, E. A., RUTLEDGE, L. T. & CALHOUN, H. D. (1958). Megimide and metrazol. A comparison of their convulsant properties in man and cat. *Electroenceph. clin. Neurophysiol.*, **10**, 719–723.

SACKS, J. & GLASER, N. M. (1941). Changes in susceptibility to the convulsant action of metrazol. *J. Pharmac. exp. Ther.*, **73**, 289–295.

SCHOEN, R. (1926). Beitraege zur Pharmakologie der Körperstellung und der Labyrinthreflexe. XXII. Mitteilung: Hexeton und Cardiazol. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **113**, 257–274.

SILLE, G. & SAYERS, A. (1967). Motor convulsions and EEG during maximal electroshock in the rat. *Int. J. Neuropharmac.*, **6**, 169–174.

STARZL, T. E., NIEMER, W. T. & FORGRAVE, P. R. (1953). Cortical and subcortical electrical activity in experimental seizures induced by metrazol. *J. Neuropath. exp. Neurol.*, **12**, 262–276.

STEG, G. (1964). Efferent muscle innervation and rigidity. *Acta physiol. scand.*, **61**, suppl. 225, 5–53.

STRAUSS, H. & LANDIS, C. (1938). Metrazol convulsions and their relation to the epileptic attack. *Proc. Soc. exp. Biol. Med.*, **38**, 369–370.

STRAUSS, H., LANDIS, C. & HUNT, W. A. (1939). The metrazol seizure and its significance for the pathophysiology of the epileptic attack. *J. nerv. ment. Dis.*, **90**, 439–452.

STRECKER, E. A., ALPERS, B. J., FLAHERTY, J. A. & HUGUES, J. (1939). Experimental and clinical study of effects of metrazol convulsions. *Arch. Neurol. Psychiat., Chicago*, **41**, 996–1003.

TEN CATE, J. (1950). Spontaneous electrical activity of the spinal cord. *Electroenceph. clin. Neurophysiol.*, **2**, 445–451.

TOMAN, J. E. P., SWINYARD, E. A. & GOODMAN, L. S. (1946). Properties of maximal seizures, and their alteration by anticonvulsant drugs and other agents. *J. Neurophysiol.*, **9**, 231–239.

TRUITT JR., E. B., EBERSBERGER, E. M. & LING, A. S. C. (1960). Measurement of brain excitability by use of hexafluordiethyl ether (indoklon). *J. Pharmac. exp. Ther.*, **129**, 445–453.

VERNADAKIS, A. & WOODBURY, D. M. (1965). Effects of diphenylhydantoin on electroshock seizure thresholds in developing rats. *J. Pharmac. exp. Ther.*, **148**, 144–150.

VERNADAKIS, A. & WOODBURY, D. M. (1969). The developing animal as a model. *Epilepsia*, **10**, 163–178.

VIANNA DIAS, M. U. (1954). Estudo miográfico das convulsões epileptiformes produzidas pela excitação da córtex cerebral. *An. Acad. Bras. Ciênc.*, **26**, 475–529.

VULPE, M., CALDWELL, D. F. & RODIN, E. (1963). Ontogenesis of megimide seizures in the rat. *Exp. Neurol.*, **8**, 350–360.

WEBB, D. L. & DAVIS, W. M. (1964). Maturational changes in the response of rats to a convulsant drug. *Arch. int. Pharmacodyn.*, **150**, 177–185.

WHITEHEAD, R. W., NEUBÜRGER, K. T., RUTLEDGE, E. K. & SILCOTT, W. L. (1940). Pharmacologic and pathologic effects of repeated convulsant doses of metrazol. *Am. J. med. Sci.*, **199**, 352–359.

WOODBURY, L. A. & DAVENPORT, V. D. (1952). Design and use of a new electroshock seizure apparatus, and analysis of factors altering seizure threshold and pattern. *Arch. int. Pharmacodyn.*, **92**, 97–107.

WOODBURY, D. M. & ESPLIN, D. W. (1959). Neuropharmacology and neurochemistry of anticonvulsant drugs. *Res. Publ. Ass. nerv. ment. Dis.*, **37**, 24–56.

ZETLER, G. & MOOG, E. (1958). Die Bulbocapnine-Katatonie, ihre Synergisten und Antagonisten. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **232**, 442–458.

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