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AN AVIAN MODEL OF GENETIC REFLEX EPILEPSY

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INTRODUCTION

Reflex epilepsy was first discovered experimentally. Increasing the excitability of a particular sensory cortex while stimulating the corresponding peripheral sensory receptors results in partial myoclonus, which may or may not be followed by self sustained generalized convulsions (1, 6, 32) synchronous with an electroencephalographic (EEG) afterdischarge (42). Genetic reflex epilepsy (GRE) was later discovered in rats by Morgan and Morgan (31). They found a particular strain of rats, in which complex, high frequency auditory stimulation precipitates convulsions. Successively, it was observed that visual stimulation with repetitive flashes of light produced myoclonus and convulsions in familiarly-predisposed humans (44). Visually induced epileptic manifestations have also been found in Baboons *Papio papio* (20, 21, 22) living in a well-defined habitat; genetic transmission has also been suggested in this case (3). Some of the *Papio papio* also show paroxysmal myoclonus induced by surprise or movement (28). Finally, in 1970 Crawford (8) described an individual chicken of the Fayoumi strain affected by GRE, seizures being induced by complex sensory stimulation and particularly by photogenic stimulation. Such epileptic chickens carry an autosomal recessive mutation; the homozygotes exhibit epileptic fits (Fepi for Fayoumi epileptic) while the heterozygotes (Fhtz) do not.

GRE is a type of epilepsy in which a particular sensory stimulus (the "epileptogenic stimulus") evokes epileptic manifestations only in genetically predisposed subjects. Theoretically, the epileptogenic stimulus can be of any sensory modality, each strain or species being more or less sensitive to a particular one. The epileptic manifestations are variable both within and among individuals, going from a simple paroxysmal electrical discharge, with or without motor disorders, to generalized convulsions with or without electrical discharges (34).

With regard to the genetic transmission of the syndrome, no specific genes have been identified so far. The term "predisposition" underlines our ignorance about spe-

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Dedication: The Authors of this article dedicate their recent work in honor of Professor Ottavio Pompeiano (particularly the first author who has been his pupil and collaborator, and remains his friend).

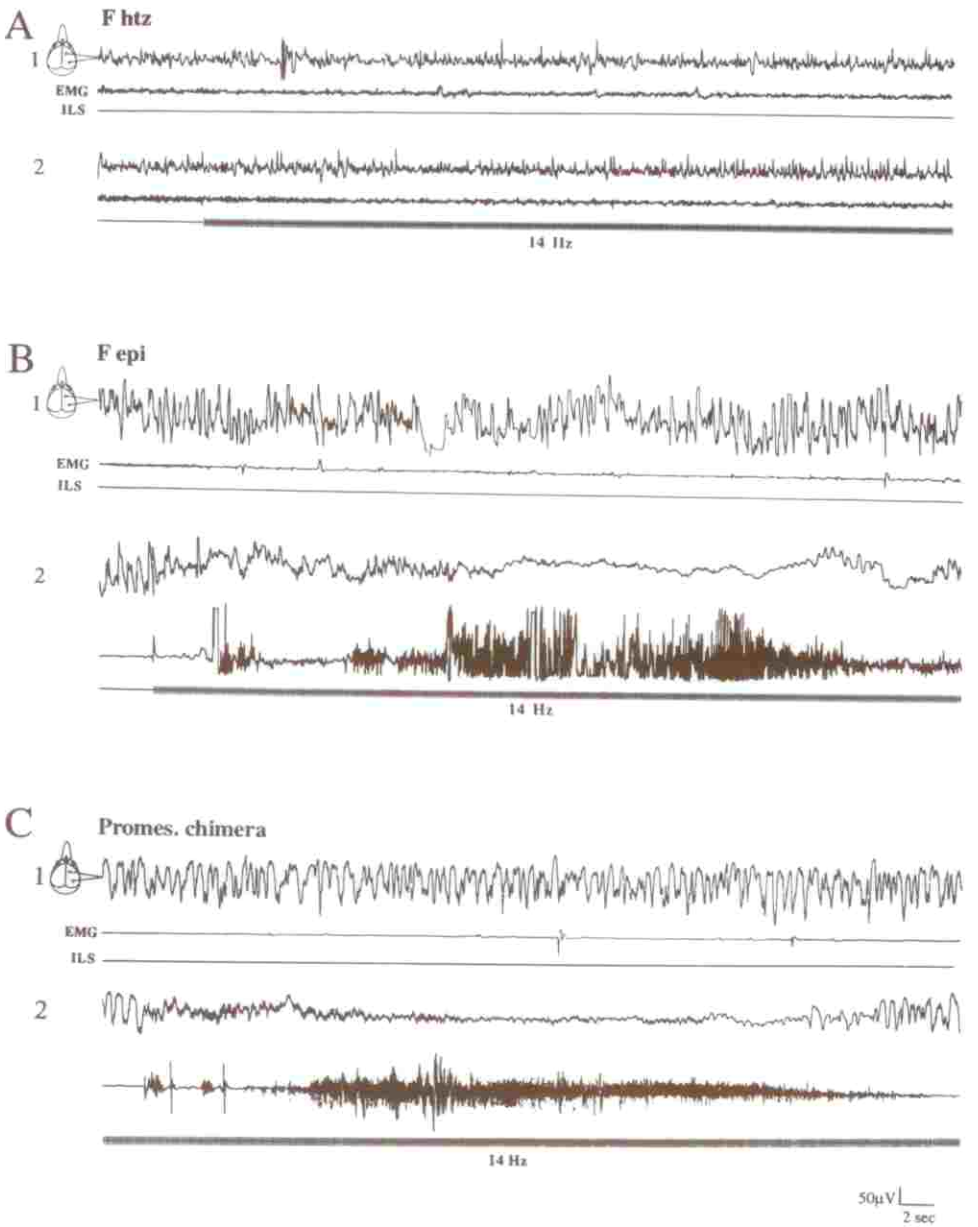

Fig. 1. - *Effects of intermittent light stimulation on Fayoumi chickens and chimeras.*

Recordings of the EEG and the neck muscle EMG in a Fayoumi heterozygous (Fhtz) (A), a Fayoumi homozygous (B) and a group III chimera (C) at rest (1) and during intermittent light stimulation (ILS) (2). Note the ILS-induced seizures in B and C but not in A. Modified from Ref. 17.

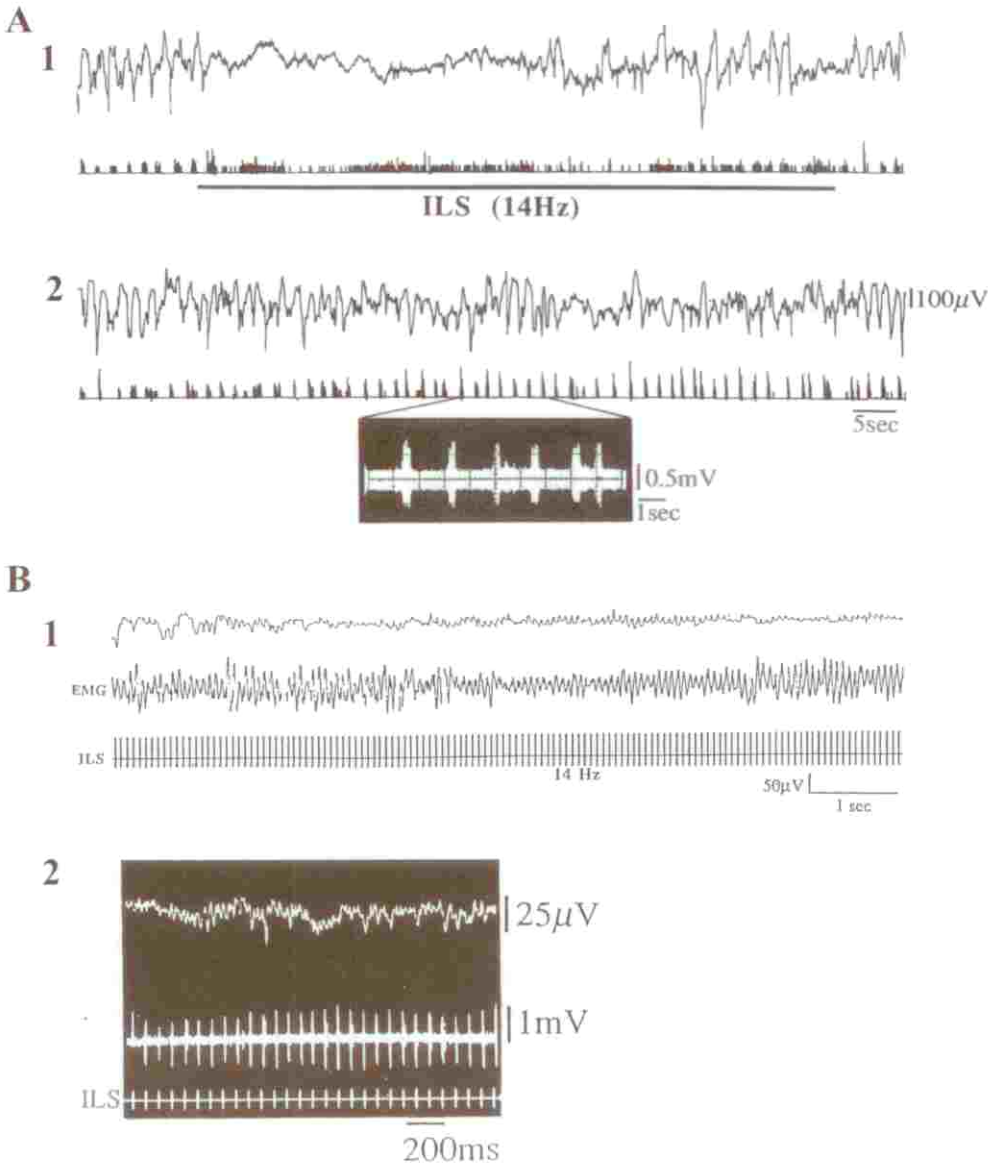

Fig. 2. - *Extracellular unit activities of prosencephalic and mesencephalic neurones in Fepi and chimeras.*

A: Simultaneous recording of EEG (upper trace) and integrated unit activities (lower trace) in the prosencephalon of a flaxedil-paralyzed Fepi during (1) and after (2) ILS-induced ictal EEG arousal. Note the bursting activity of the prosencephalic neurones at rest. B1: EEG and neck muscle EMG recorded in a group III chimera showing persistent neck myoclonus during ILS. B2: Simultaneous recording of the EEG (upper trace) and of a unit in the mesencephalon (middle trace) of a paralyzed group III chimera showing ILS-induced paroxysmal bursting activity of the neurone. Note the normal EEG activity in 1 and 2. Modified from Ref. 14.

Group	Type of chimera (Pro, Mes, Rho; Tel, Di, Mes, Met, Mye; 12-somite stage)	Number of cases	EEG Paroxysms	ILS				ISS				Type of transfer
				Phase			DF	Phase			D	
				A		B		A		B		
				IA	My	Co		IA	RF	Co		
I		3	+	+	—	—	+	+	—	—	+	Transfer of EEG activity
II		1	+	+	+	+	+	+	+	+	+	Total transfer of epileptic phenotype
		3	+	+	+	+	+	+	+	+	+	
III		6	—	—	+	—	—	—	+	+	—	Transfer of ILS-induced neck myoclonus and ISS-induced running fits and generalized convulsions
		2	—	—	+	—	—	—	+	+	—	
		1	—	—	+	—	—	—	+	+	—	
IV		2	—	—	—	—	—	—	—	—	—	No epileptic manifestations
C		1	—	—	—	—	—	—	—	—	—	
		3	—	—	—	—	—	—	—	—	—	

Fig. 3. - *Building a brain chimera.*

A: Schematic representation of the exchanged parts of the neuroepithelium between two chick embryos at the 12-somites stage. The constrictions separating the encephalic vesicles are used as landmarks to limit the graft. In the experiment presented here, the mesencephalic and metencephalic vesicles were excised from a Fepi embryo and grafted in the place of the same structures in a normal chick embryo. The resulting chimera shows the feather pigmentation of the Fepi donor (white) in the graft area, since the neural crest from which pigment cells arise was grafted with the Fepi brain vesicles. Abbreviations for this and the following figure: Co: convulsions, D: EEG desynchronization, DF: EEG desynchronization and flattening. Di: diencephalon, IA: ictal arousal, Mes: mesencephalon, Met: metencephalon, My: myoclonus, Mye: myelencephalon, Pro: prosencephalon, RF: running fit, Rho: rhombencephalon, Tel: telencephalon.

vulsions, although these occurred less frequently then in group II chimeras. In fact, 80% of all sound presentations precipitate convulsions in group II chimeras, versus 30% in group III chimeras. It should be pointed out that the mesencephalon was the minimum amount of Fepi grafted neuroepithelium capable of transferring epileptic symptoms in this group of chimeras.

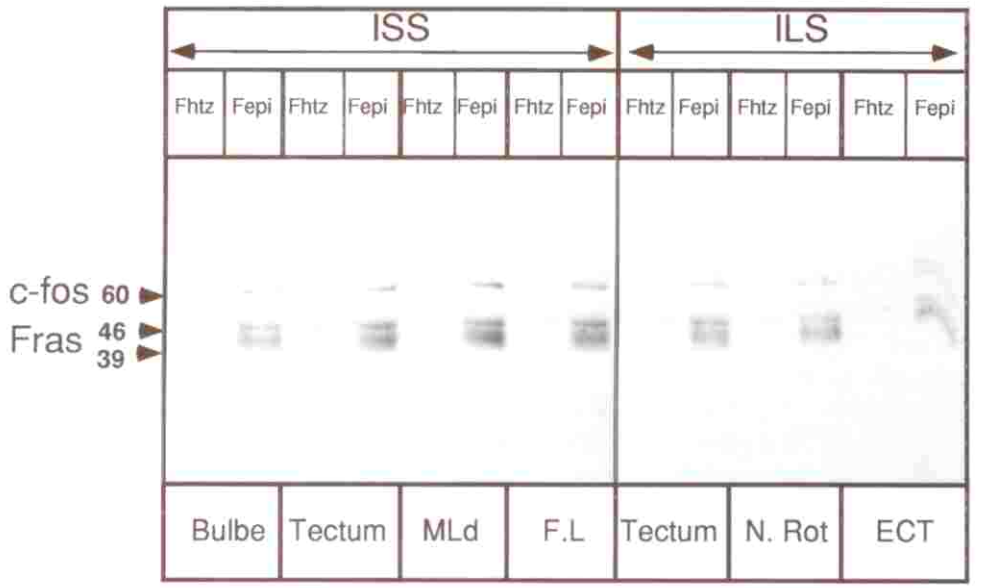

Fig. 5. - *Expression of c-fos in the sensory pathways of the Fhtz and Fepi chickens after epileptogenic stimulation.*

Western blot obtained from two Fhtz and two Fepi sacrificed 3 hours after audiogenic (ISS) and photogenic (ILS) stimulation (seizures were induced in the Fepi and not in the Fhtz). Samples were selected from the same structures in Fhtz (1 to 7) and Fepi (1' to 7'). In the animals submitted to ISS, the samples included primary acoustic relay nuclei of the brainstem *(nucleus coclearis)*, the tectum (deep layers), the *MLd. (nucleus mesencephalicus lateralis, pars dorsalis)* and the FL (field L in the telencephalon). In the animals submitted to ILS, the samples included the visual relays of the tectum (superficial layers), the N. Rot *(nucleus rotundus* in the diencephalon) and the Ectostria (ectostriatum in the telencephalon). Note the increased expression of Fos (60 KD) and FRAs (46 and 39 KD) in the two Fepi chickens.

The last relevant result (41, 17, 4) is that the chimeras having the neuroepithelium caudal to the mesencephalon exchanged (group IV in Figure 4), never produced seizures. This result is consistent with several alternative interpretations. The possibility exists that these parts of the brain do not carry the genetic defect. Alternatively, the genetic defect might be present, but the functions of those brain structures would not be adequate for induction of the epileptic symptoms. As a corollary this would mean that the genetically determined potential to become a Fepi prevails in the anterior part of the brain, not in the posterior part. Finally, these results also show that the motorneurones do not need to have an epileptic genotype to transmit motor seizures.

What we learned from the brain chimeras is that the epileptic phenotype can be transferred in normal chickens totally or partially, depending on the territory of the Fepi neuroepithelium that was grafted. The Fepi prosencephalon by itself only transfers the interictal and ictal electrographic signs of Fepi GRE and therefore only carries a general seizure susceptibility. The mesencephalon transfers neck myoclonus or RF, which are stimulus-locked, and therefore carries only a specific sensory-motor seizure susceptibility. It is through the conjunction of both the prosencepalic

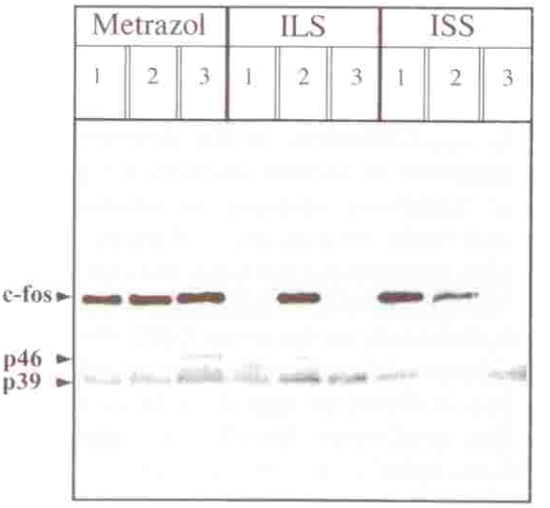

Fig. 6. - *Expression of c-fos after Metrazole-, ILS-, and ISS-induced seizures.*

Western blot obtained from three Fepi sacrificed 3 hours after seizures induced by Metrazole, or by audiogenic (ISS) or photogenic (ILS) stimulation. Samples were taken from the same structures in the three animals. 1: brain stem, 2: mesencephalon (tectum and tegmentum), 3: hippocampus. See text for explanations of the differential expression of c-fos in the three animals.

follows: a) all the structures tested display intense c-fos expression in the case of metrazole-induced seizures, which are known to involve the entire brain (25) [2]; b) intense c-fos expression is obtained only in the mesencephalon with light-induced seizures; c) with sound-induced seizures, intense c-fos expression is obtained, not only in the mesencephalon, but also in the bulbar region; d) FRAs are expressed to a lesser extent in all the samples studied, whatever the type of seizure obtained, therefore indicating a non specific effect [3].

Thus, metrazole-induced seizures are distinguished from the GRE. Photogenic GRE appears to have a localization distinct from audiogenic GRE, with only the latter showing an intense c-fos expression in the bulbar sample. This is interpreted as due to the sound-induced hyperactivity of the neurones of the primary sensory relays lying in the bulbar region, whereas the primary visual relays lie in the mesencephalon.

Finally, whatever the epileptogenic stimulus used, a common intense c-fos expression is observed in the mesencephalic samples which include the visual as well as the tecto-spinal relays. Therefore c-fos expression appears to be more intense in the animals having light-induced seizures than sound-induced seizures.

Altogether, the results obtained in the c-fos expression experiment are perfectly compatible with those obtained in the experiments using Fepi chimeras. They confirm that Fepi photogenic and audiogenic seizures have partially distinct localization. They also confirm once more the mesencephalic localization of the motor seizures initiator for Fepi GRE.

[3] Increase of FRA expression persists much longer (days) then Fos expression (hours) (40). Seizures were induced six hours before sacrificing the animal. This is the optimal time for the visualization of seizure-induced Fos expression. FRA expression has a much longer persistence time (40), and depends not only on the seizure history of the previous days but also on the net activity due to other uncontrollable factors during the previous days.

SUMMARY

The Fayoumi strain of chickens (Fepi) carries a recessive autosomal gene mutation in which homozygotes are afflicted with a photogenic and audiogenic reflex epilepsy.

Seizures consist of stimulus-locked motor symptoms followed by generalized self sustained convulsions. EEG recordings show spikes and spike and waves patterns at rest which are suppressed during seizures and replaced by a desynchronized pattern of activity. Neurones of the prosencephalon discharge in bursts at rest, while neurones of the mesencephalon are bursting during seizures.

Living neural chimeras were obtained by replacing specific embryonic brain vesicles in a normal chicken embryo with equivalent vesicles from a Fepi donor. These chimeras show that the epileptic phenotype can be totally or partially transferred from the Fepi to the normal chickens. Total transfer of photogenic and audiogenic seizures was obtained by substitution of both the prosencephalon and mesencephalon, while substitution of the prosencephalon alone resulted in transfer of interictal paroxysmal activity and substitution of the mesencephalon alone resulted principally in transfer of ictal motor symptoms.

Increased expression of the c-fos protooncogene, as revealed by the western blot technique, confirmed the distinct encephalic localizations of the symptoms of the photogenic and audiogenic reflex epilepsy of the Fepi shown with the methods of electrophysiology and brain chimeras. We conclude that the Fepi is a good model of brain stem reflex epilepsy and suggest that the brain stem is a generator of some other animal and human genetic reflex "epileptic syndromes".

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