Progress in Neurobiology Vol. 34, pp. 185 to 196, 1990
Printed in Great Britain.

0301-0082/90/$0.00 + 0.50

STEREOTAXIC ATLAS FOR THE LIZARD *GALLOTIA GALLOTI*

J. M. DEL CORRAL, A. MIRALLES, M. C. NICOLAU, B. PLANAS and R. V. RIAL*
Department of Biology and Health Sciences, University of the Balearic Islands, 07071 Palma de Mallorca, Spain

(*Received* 9 *October* 1989)

CONTENTS

1. INTRODUCTION

Atlases for the brains of nonmammalian animals, useful for stereotaxic surgery, are scarce to our knowledge. Atlases are available for a few species of fishes (Peter *et al.*, 1975; Peter and Gill, 1975; Billard and Peter, 1982), the frog (Netchitailo *et al.*, 1987), the iguana (Distel, 1976), the chick (Kuenzel and Tienhoven, 1982) and the pigeon (Karten and Hodos, 1967).

The lower vertebrate brain is being increasingly used as a simplified model of the mammalian brain in morphological, physiological, evolutionary, comparative and pharmacological studies. In particular, the lizard *Gallotia galloti* is actively used in investigations in all these fields (Molowny *et al.*, 1972; Rial and González, 1978; De Vera *et al.*, 1986; Gómez *et al.*, 1988). However, up to now, all physiological studies have been restricted to cortical areas due to the incomplete knowledge of the subcortical structures.

For stereotaxic purposes, the reptilian brain differs from the mammalian one: one cannot talk of a standard body size, due to the continuous growth of these animals. This raises the need for the design of a system of relative units which would allow the use of animals of different body size. In this respect, the brain of the reptile seems to grow with the body length (Platel, 1972). Also, it must be noted that the reptilian brain does not fill the skull in the same way as the mammalian brain does, but is placed, leaving some space, in the cranial cavity, surrounded by a strong membrane, the dura mater, which makes access to deep centers in the brainstem very problematic, unless a high degree of precision can be achieved.

This paper deals with the design of a stereotaxic technique and the description of brain structures in a stereotaxic atlas for the lizard *Gallotia* (formerly *Lacerta*) *galloti*.

2. MATERIALS AND METHODS

2.1. ANIMALS

For the whole study, 135 specimens of the canarian lizard (*Gallotia galloti*) have been used, with body length (nose tip to cloacal opening) ranging from 66 to 130 mm. After capture, the animals were maintained in terrariums which simulated their natural habitat, with food and water continuously available.

On each animal, measurements were taken for each one of the most prominent head sutures. Statistical data were derived for the distribution of the individuals respective to the measured parameters, and correlation indexes between body size and the length of cephalic sutures were calculated. As a result, the best correlation index (CI = 0.87) was found between the individual size and the length of the interfrontoparietal suture (IFPS), a value concordant with previous studies (Bolaños, 1976). In the individuals considered, the length of IFPS varied from 3.0 to 6.8 mm. No significant allometries were found between head sutures and body size (the head growth was linearly correlated to body growth).

*Send correspondence to: R. Rial, Department of Biology and Health Sciences, University of the Balearic Islands, 07071 Palma de Mallorca, Spain.

The lack of allometry, and the good correlation found allowed the assumption (not yet proved) that there would also be a good correlation between the IFPS length and brain size, in spite of some minor allometries found between different brain regions in other reptiles studied (Bauchot and Platel, 1971). Thus, the sutures between head plates were used as reference points for coordinates of brain structures, and the length of the IFPS divided by 20 has been used as a relative unit in the design of the stereotaxic atlas.

2.2. APPARATUS

A head holder, easily adaptable to most of the normal rat stereotaxic apparatus, was designed clamping the upper jaw and the otic fossa. The skull of reptiles is very complex. Only the telencephalon is below a single bone layer, the lower brainstem zones being protected under a complex system of occipital plates (Fig. 1). For this reason, the stereotaxic plane was orientated at an angle of 38° with the horizontal plane. When the skull is set in this position, the parietal bones rest at an angle of 53° with the horizontal plane and most of the lower brainstem regions are easily accessible through a single hole made in the parietal bones, in a similar form that Distel (1976) did for the iguana. The center of the parietal eye was used as the origin of coordinates.

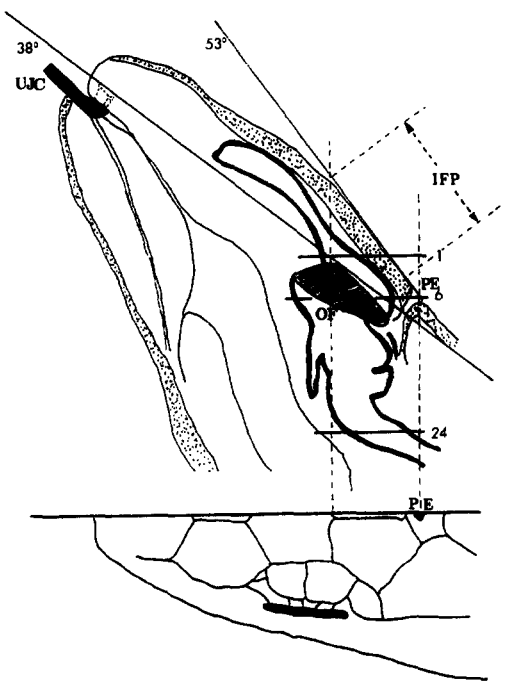

FIG. 1. Schematic drawing of the skull showing the main planes and the placement of the first (1), the sixth (6) and the last (24) pages of the atlas. The brain regions are shown in correspondence with the interfrontoparietal suture (IFPS). U.J.C. = Upper jaw clamp. O.F. = otic fossae (in dark). P.E. = parietal eye.

2.3. MARKING LESIONS

In 14 animals lesions were made at arbitrary coordinates to prove the accuracy of the technique and atlas. These animals were chosen with different body sizes (88.8 to 123.9 mm), anesthetized with urethane and lesions were made through holes perforating the parietal bone. The introduction and immediate retraction of a 0.25 mm θ pin, rendered an easily perceptible trajectory in transverse sections.

2.4. HISTOLOGICAL TECHNIQUES

The animals, under urethane anesthesia, were perfused through the cardiac ventricle with a Krebs–Ringer solution adapted to reptiles (Umbreit *et al.*, 1959) followed by buffered formalin (10% formaldehyde in phosphate buffer, pH 7.2) and then decapitated; the head remained in the same fixative solution for at least 48 hr. Some skulls were treated in a Castro's decalcifying solution for 24 hr. The brain of the first ones and the whole heads of the last ones were embedded in paraffin, after which each brain was cut into transverse serial sections, 30 μm thick, distributed in complete parallel sets that were stained with toluidine blue (Nissl). All stained sections were photographed and superimposed to a Buerker hematometric camera to provide exact measures. Drawings were made at 40× magnification with a camera lucida.

2.5. BRAIN RECONSTRUCTION

To accomplish brain reconstruction from the histological sections, it was necessary in the first step to evaluate the shrinkage of the cerebral tissues caused by fixation and inclusion procedures. This was made by choosing pairs of animals with a very high likeness in their physical parameters. One of each pair was used to make the atlas, and the other was used as a pattern to localize the sections obtained from the first one. The fresh head of this animal was cut through a medial rostrocaudal plane, and one of these half heads was mounted in the stereotaxic apparatus to obtain distances between significant brain regions and cephalic sutures. These distances were used as real measures, irrespective of the actual measures of the fixed-stained brain. For instance, in one case the distance between the end of the telencephalon to the beginning of the cerebellum was found to be 3000 μm in the fresh brain; between these two points there were 96 sections in the fixed-stained brain, each section being approximately 31 μm thick (3000/96 = 31.25). One from every ten sections was selected to make the stereotaxic atlas, giving a distance between two atlas pages of approximately 310 μm, which equals the IFPS length divided by 20. This fraction was selected as a relative unit in the atlas. Also, to assign a page number to each section, its location relative to the parietal eye in relative units was determined. The results obtained with this method were contrasted with those obtained from the decalcified whole heads.

2.6. NOMENCLATURE

The terms used for identification of brain structures were selected from topologic and architectonic information on *Gallotia galloti* studies of Molowny *et al.* (1972), Martin Trujillo *et al.* (1975), Regidor (1978), López-García *et al.* (1983) and from studies on other species (Distel, 1976).

3. RESULTS

The atlas obtained is presented in Figs 2 to 7 with abbreviated symbols to explain the main recognized regions. The list of used abbreviations is as follows:

Abbreviation	Structure	Location
a.c.a.	Nucleus anterior centralis amigdalae	4–6
Acc	Nucleus accumbens	2–5
a.c.p.	Nucleus posterior centralis amigdalae	7
ADH	Nucleus dorsalis hypothalami	11–12
ADVR	Hyperstriatum anterior (anterior dorsal ventricular ridge)	2–6
a.l.d.	Nucleus laterodorsalis amigdalae	7–9
ALH	Nucleus lateralis hypothalami	9–14
a.l.v.	Nucleus lateroventralis amigdalae	7–10
APL	Nucleus praeopticus lateralis	8
APM	Nucleus praeopticus medialis	8
AT	Area triangularis	10–11
AVM	Nuclei ventralis thalami	14
CA	Anterior commissure	7–8
CbM	Nucleus cerebelaris medialis	21
CG	Griseum centrale	13
CoP	Commissura posterior	15
c.p.a.	Commissura palii posterior	9
CT	Commissura tectalis	14–16
D	Cortex dorsalis	1–10
DLP	Nucleus dorsolateralis posterior	11
DM	Nucleus dorsomedialis	10–12
DR	Decussatio retroinfundibularis	16
DSV	Decussatio supraoptica ventralis	11
DT	Decussatio tegmenti	21
Dvl	Nucleus Deiters, pars ventrolateralis	24
DVRc	Hyperstriatum posterior (posterior dorsal ventricular ridge)	2–6
FLM	Fasciculus longitudinalis medialis	22–24
FPLv	Fasciculus prosencephali lateralis, pedunculus ventralis	13–17
FPM	Fasciculus prosencephali medialis	14
GLd	Corpus geniculatum lateralis, pars dorsalis	11
HTL	Hypothalamus lateralis	9–11
Imc	Nucleus isthmi, pars magnocellularis caudalis	20–22
IP	Nucleus interpeduncularis	19–21
Ipc	Nucleus isthmi, pars parvocellularis	20–22
IS	Nucleus interstitialis, fasciculus longitudinalis medialis	15
L	Cortex lateralis	1–8
La	Nucleus lateralis	24
LAH	Nucleus lateralis hypothalami	9–14
LLd	Nucleus lemnisci lateralis, pars dorsalis	22
LMe	Nucleus lentiformis mesencephali	12
LN	Corpus geniculatum lateralis (neuropile)	11–13
LS	Lemniscus spinalis	24
M	Nucleus medialis	11–12
M1	Cortex medialis, pars medialis	1–10
M2	Cortex medialis, pars magnocellularis dorsalis	1–10
MC	Corpus geniculatum lateralis, pars ventralis	11–13
MNv	Nucleus mesencephalicus nervii trigemini	14–16
Mvv	Nucleus motorius nervii trigemini	23
NH	Nucleus habenulae	10
NM	Corpus mamillaris	15
NO	Nucleus ovalis	10
NS	Nucleus sphaericus	7–10
NSv	Nucleus tractus spinalis nervii trigemini	24
ntol	Nucleus tractus olfatorius lateralis	6
NIV	Nucleus nervii trochlearis	18–24
nIV	Nervus trochlearis	18–24
nIII	Nervus oculomotoris	18

Continued

Abbreviation	Structure	Location
nVm	Nervus trigeminus, radix motora	23
nVIId	Nervus octavus, pars dorsalis	24
nVIIv	Nervus octavus, pars ventralis	24
nVIIm	Nervus facialis, radix motora	24
OMv	Nucleus oculomotorius, pars ventralis	17–21
OS	Nucleus olivaris superior	24
OT	Nucleus opticus tegmenti	16–17
PD	Nucleus geniculatus lateralis, pars dorsalis	10
PMdl	Nucleus profundus mesencephali, pars dorsolateralis	18–19
PMl	Nucleus profundus mesencephali, pars lateralis	16–19
PMm	Nucleus profundus mesencephali, pars medialis	14–17
PMr	Nucleus profundus mesencephali, pars rostralis	18
PrV	Nucleus sensorius principalis nervii trigemini	23
PT	Pallial thickening	4–5
PV	Nucleus paraventricularis	9
PVH	Nucleus paraventricularis hypothalami	9–15
QO	Chiasma opticum	4–10
R	Nucleus rotundus	12–13
RM	Nucleus reticularis medialis	23–24
RMS	Nucleus magnocellularis superior raphes	22–24
ROB	Radix optica basalis	16
RPS	Nucleus parvocellularis superior raphes	22
RS	Nucleus reticularis superioris	22–23
Ru	Nucleus ruber	16–19
SAC	Stratum album centrale	12–17
SAP	Stratum album periventriculare	12–20
SD	Corpus striatum, pars dorsalis	1
SGC	Stratum griseum centrale	12–17
SGF	Stratum griseum et fibrosum superficiale	12–17
SGP	Stratum griseum periventriculare	12–20
SGr	Stratum granulare cerebelli	17–21
SMo	Stratum moleculare cerebelli	16–21
SOp	Stratum opticum	14–16
SPu	Stratum Purkinje cerebelli	17–21
SP	Septum	1–8
SRt	Nucleus subrotundus	13
SV	Corpus striatum ventralis	1–6
SV1	Corpus striatum ventromedialis	2–6
SV2	Corpus striatum ventrocentralis	2–3
SV3	Corpus striatum ventrodorsalis	2–6
Ta	Nucleus tangentialis	24
TO	Tuberculum olfatorium	2–6
ToS	Torus semicircularis	18–19
TrO	Tractus opticus	11–13
TSV	Tractus spinalis nervii trigemini	24
V	Hypothalamus lateralis, pars ventralis	10
VLD	Nucleus ventrolateralis, pars dorsalis	11
VLV	Nucleus ventrolateralis, pars ventralis	12–13
VM	Nucleus ventromedialis	13
VS	Nucleus vestibularis superior	22–23

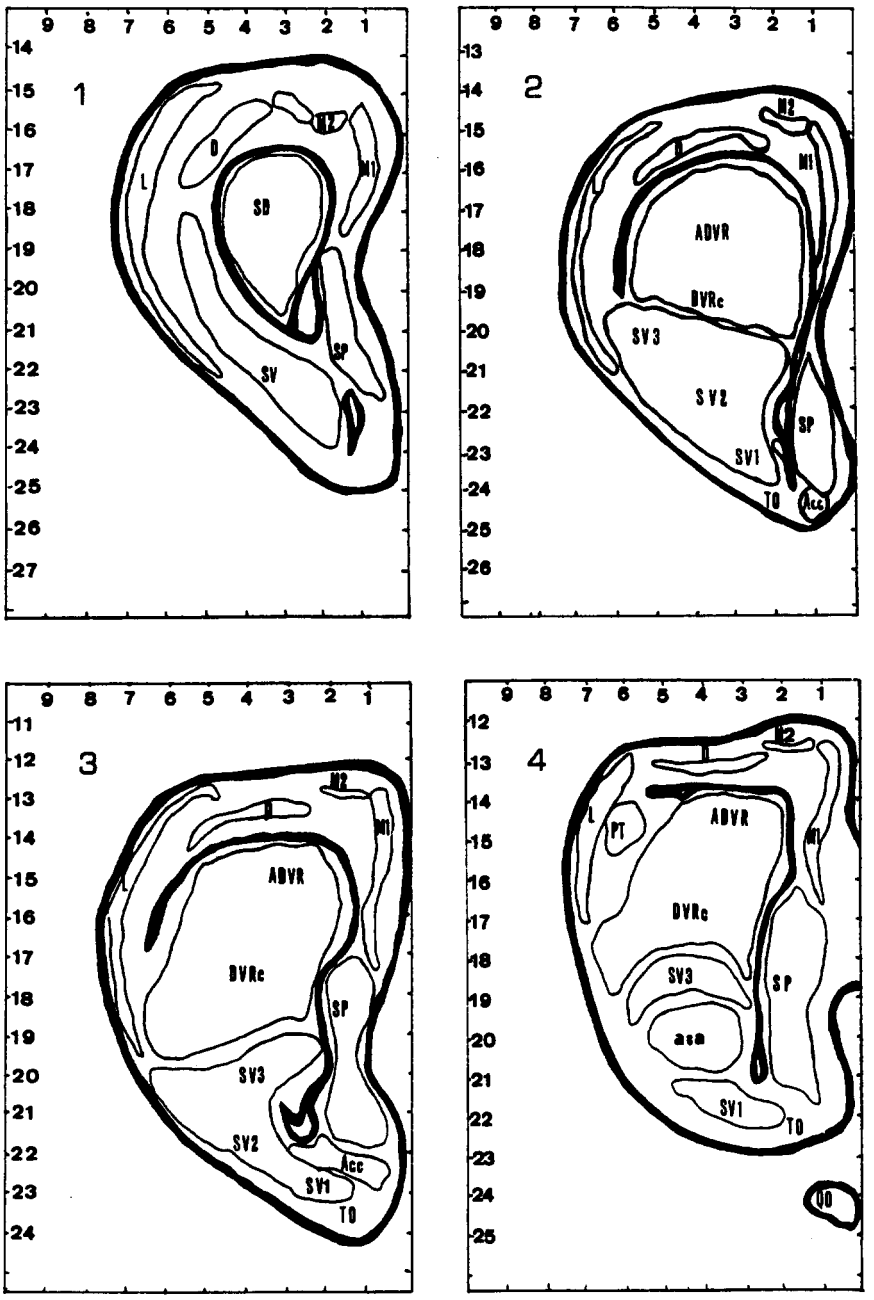

FIG. 2.

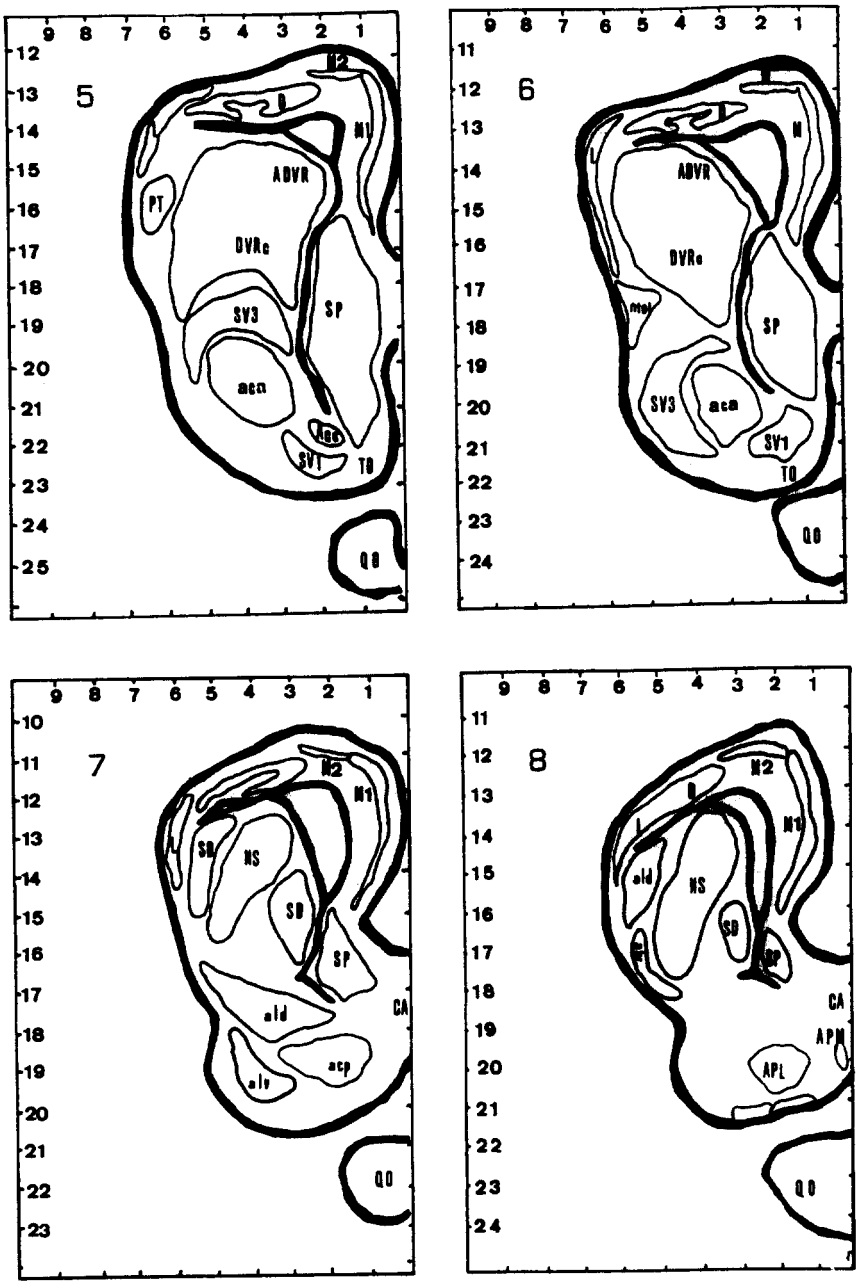
5
N2
N1
ADVR
PT
DVRc
SV3
SP
acn
Acc
SV1
TO
QO
6
N2
N1
ADVR
DVRc
SP
SV3
acn
SV1
TO
QO
7
N2
N1
SO
NS
SO
SP
ald
CA
alv
acp
QO
8
N2
N1
ald
NS
SO
SP
CA
APM
APL
QO

FIG. 3.

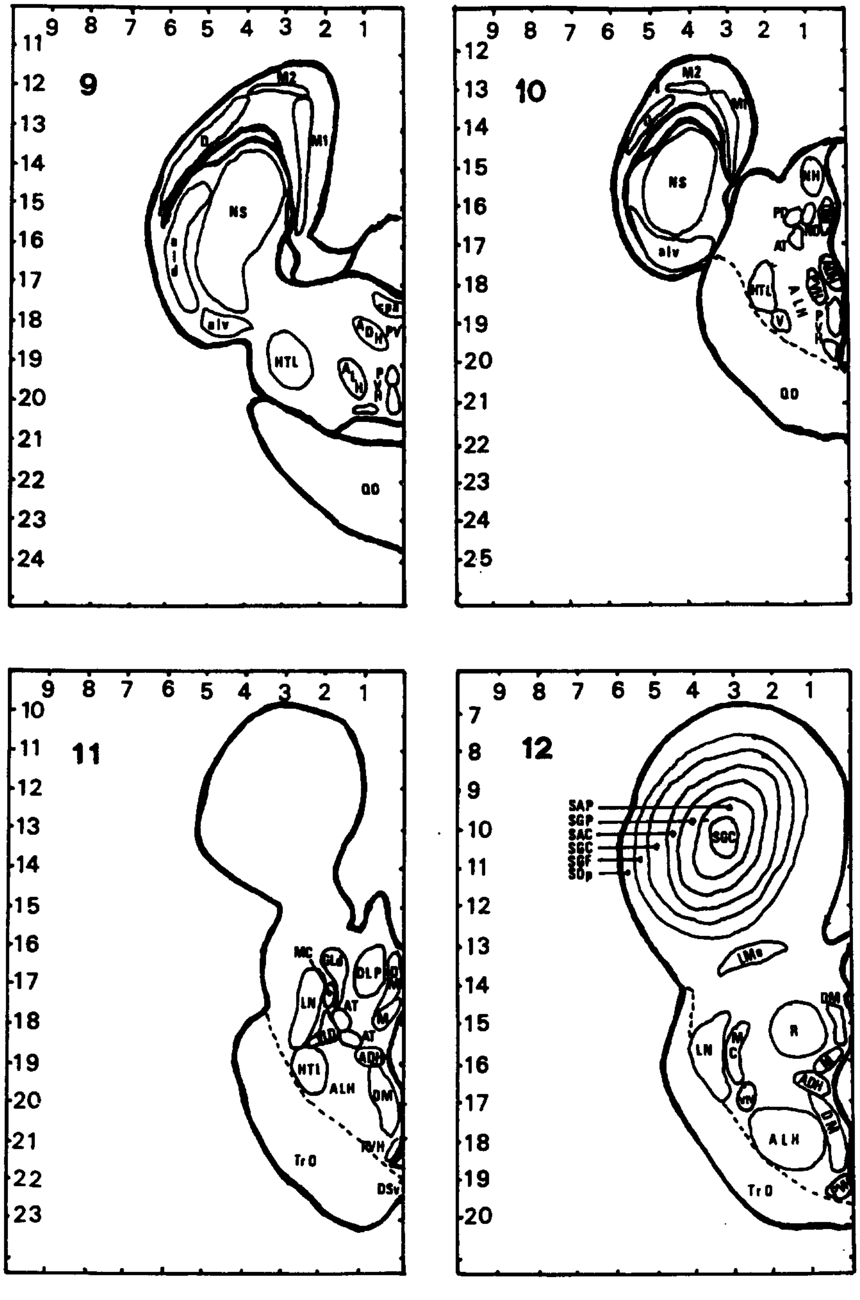
9
M2
M1
D
NS
alv
HTL
ADH
PV
ALH
QO
10
M2
M1
NS
alv
NH
AT
HTL
ALH
V
QO
11
MC
LN
DLP
AT
HTL
ALH
ADH
DM
TrO
DSv
12
SAP
SGP
SAC
SGC
SGF
SOp
SGC
LMe
LN
MC
R
DM
ADH
ALH
TrO

FIG. 4.

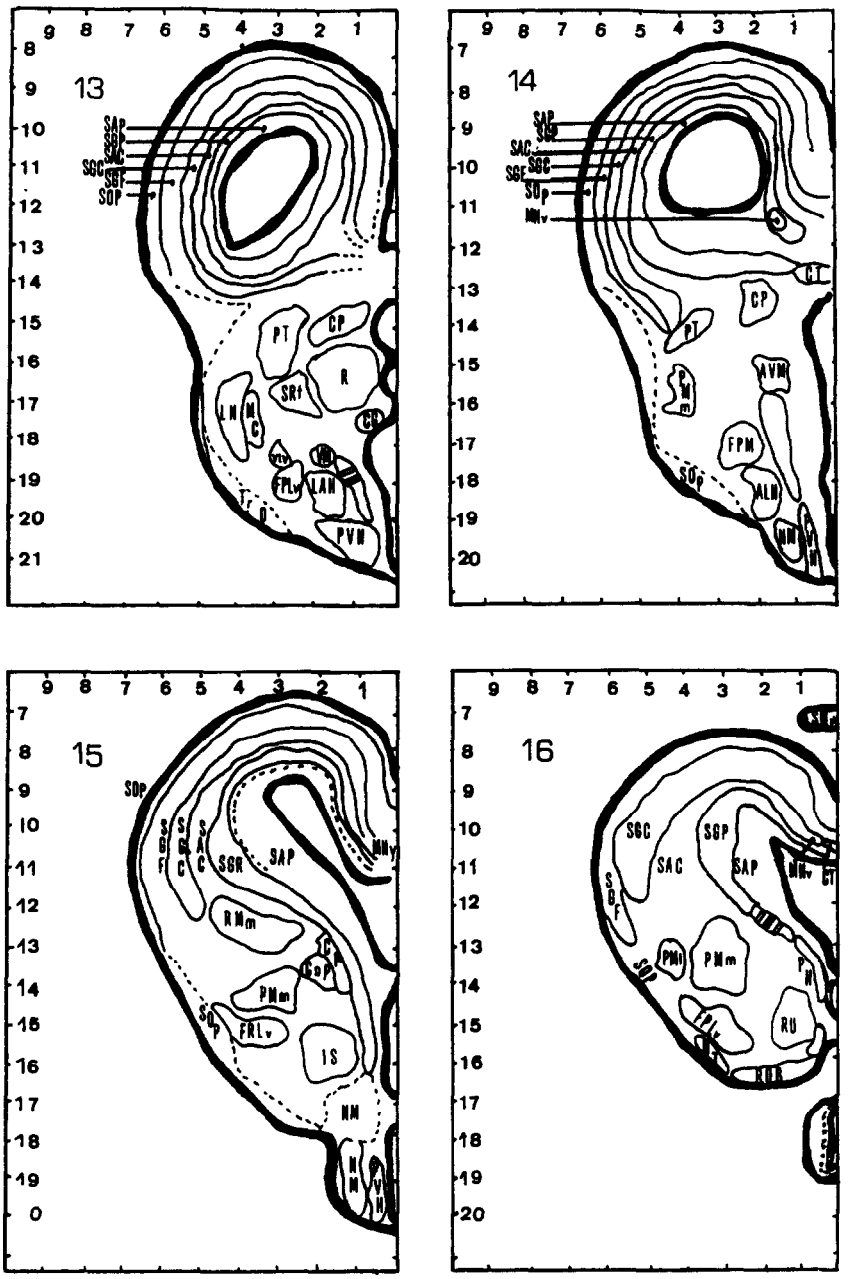
13
SAP
SGC
SOP
PT
CP
R
SRf
LN
PVM
14
SAP
SAC
SGC
SGP
SOp
MNv
CP
PT
AVM
FPM
ALM
15
SOP
SAP
SGR
RMm
PMm
FRLv
IS
MM
16
SGC
SAC
SGP
SAP
PMd
PMm
RU

FIG. 5.

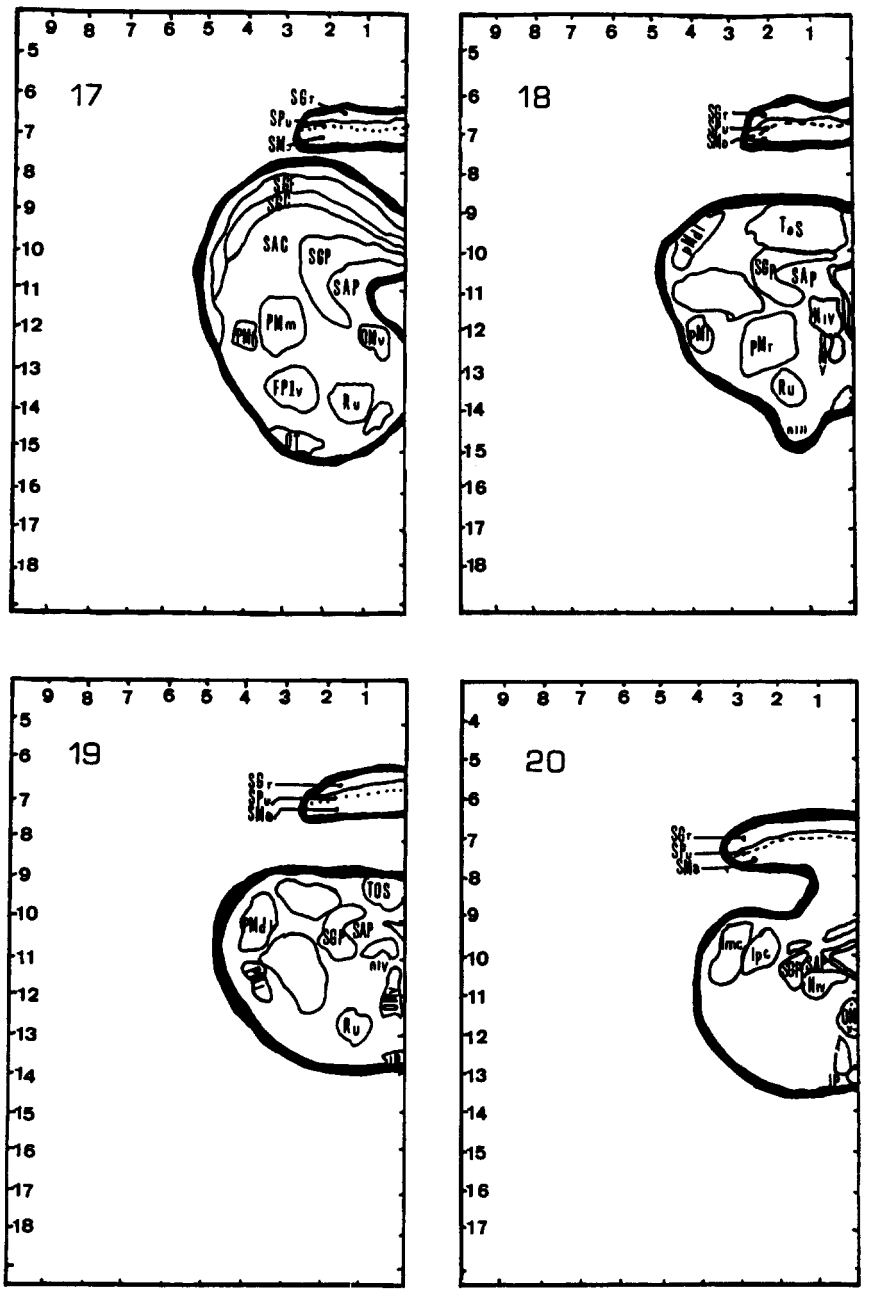

FIG. 6.

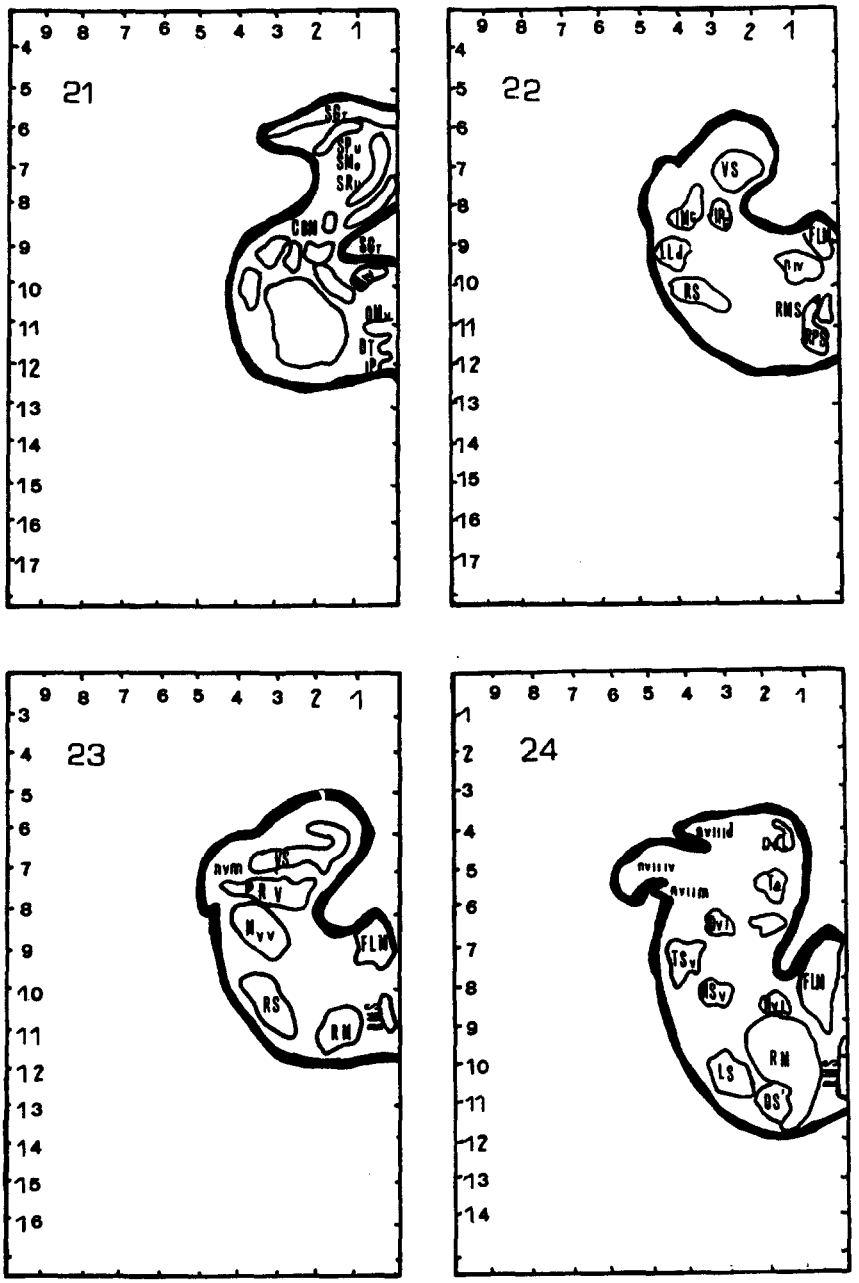

FIG. 7.

FIGS 2 to 7. Stereotaxic atlas in coronal sections with the skull placed in the position shown in Fig. 1. Each page in the atlas is separated from its neighbors by 1 IFP unit and page 6 corresponds to the section containing the parietal eye. It should be noted that the page number is the deep coordinate, and the numbers in the vertical axis of the atlas are the coordinates anterior to the parietal eye.

TABLE 1. CHARACTERISTICS OF ANIMALS AND RESULTS OF LESIONS

Animal number	Body length (mm)	IFP length (mm)	IFP/20 (unit) (μm)	IFPS Aimed coordinates Anterior	Lateral	Deep	Error found
3	115.0	5.9	295.0	10.0	3.3	22.0	1.43
8	101.0	4.45	222.5	17.9	4.4	15.0	0.82
9	96.8	4.75	237.5	16.8	4.2	15.0	1.39
10	99.6	4.5	225.0	17.7	3.8	16.0	1.41
15	121.6	6.3	315.0	12.6	6.3	16.0	1.11
26	88.8	3.9	195.0	17.9	4.1	15.0	1.29
31	107.8	5.0	250.0	16.0	4.0	16.0	1.21
32	116.5	5.5	275.0	17.8	4.3	15.0	1.16
40	114.8	5.05	252.5	17.8	4.3	15.0	1.25
45	106.6	4.8	240.0	17.9	4.1	15.0	1.10
49	123.9	4.3	215.0	16.5	4.6	15.0	1.48
50	108.9	5.05	252.5	12.5	3.9	20.0	1.33
54	116.7	6.0	300.0	10.0	3.3	24.0	1.56
62	107.5	4.9	245.0	17.9	4.0	15.0	0.96

Mean error = 1.25 IFP units.

3.1. ACCURACY OF THE METHOD

The errors found in marking lesions are presented in Table 1. It can be seen that the difference between the expected location and the actual one ranged from 183 μm (0.94 IFP units) in small animals to 333 μm (1.55 IFP units) in the largest ones. These measures are expressed as a lineal distance; if they are considered as a radius, a sphere may be postulated in which all the lesions are included around the expected location.

4. DISCUSSION

A stereotaxic atlas and technique is a tool whose validity is determined by a number of factors. As the object of this tool will be accurate access to a desired brain location, three error types may be responsible for accuracy loss: (a) errors produced by lack of precision in the technique; (b) errors produced by lack of accuracy in the atlas; and (c) errors caused by individual variability in the animals.

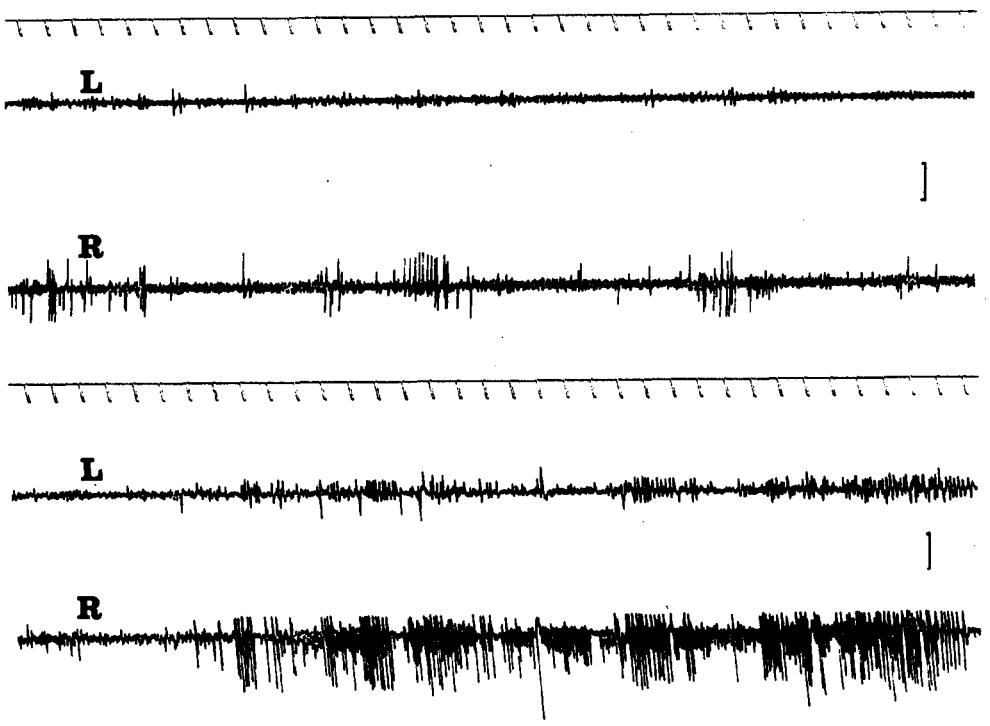

FIG. 8. Examples of EEG recordings obtained using chronic electrodes placed in the telencephalic cortex of *G. galloti* after production of the Kindling effect. The main characteristics of the signal are the abundant high frequency spindles, high voltage spiking and the presence of spontaneous electrographic seizures. Calibration marks: 5 sec and 50 μV. R: Right hemisphere; L: left hemisphere. The kindling stimulus was applied to the left hemisphere.

The cranial fixation security is the main factor in determining lack of precision in the stereotaxic technique. Though reptiles have a cranium with abundant holes and cavities, none of them is fully closed by bones, producing a setting in the apparatus which is never as rigid as it is in mammals and birds. Also, as was pointed out by Distel (1976), the electrodes often dimple the brain before penetrating into the pia or when re-entering the tissue after reaching the ventricular space, due to the flexibility of the brain tissue. In addition, for very deep placements, the flexibility of the electrode and the mechanical reaction of the brain may produce lateral deflections. However, considering the small measures of the brain in the studied animals, these last causes should account for very little of the absolute error, the lack of fixation security playing the most important role in technical errors.

Respective to the second cause of error, this will be dependent on the dimensions of the targeted area; for example, there are many nuclei in the diencephalon which in the atlas can be measured as a fraction of IFPS square unit: this will produce a very high probability of error if they are the target. Although these considerations can be made for all the known atlases, the error will be greater in smaller animals such as the lizards. In addition, a correlation has been presumed between the IFPS length and the brain measures. To prove this correlation, a larger sample of animals and thorough error calculations would be necessary.

Finally, individual variability has been the main reason for selecting the relative units method to compensate for the differences in body growth. However, this method cannot counteract differences between animals of the same size: this is another unavoidable cause of lack of accuracy.

After these considerations, the actual error figures seem to be surprisingly low, especially if they are compared with a rat's atlas in which figures in the neighborhood of 1 mm are commonly accepted. So, we feel that the technique and atlas described may be useful enough to study the function of deep brain areas and also for comparative studies on other reptiles.

REFERENCES

BAUCHOT, R. and PLATEL, R. (1971) Aspects quantitatifs de l'encephale des reptiles. *Zool. Auz. Leipzig* **187** (3/4), 147–174.

BILLARD, R. and PETER, R. E. (1982) A stereotaxic atlas and technique for nuclei of the diencephalon of rainbow trout *Salmo gairdneri*. *Repr. Nutr. Dev.* **22**(1A), 1–26.

BOLAÑOS, A. (1976) Primera aproximación a una relación entre coordenadas externas en placas cefálicas y centros cerebrales en *Lacerta galloti*. Licenciature Thesis, La Laguna University, Spain.

DE VERA, L. and GONZÁLEZ, J. (1986) Effect of body temperature on the ventilatory responses in the lizard *Gallotia galloti*. *Resp. Physiol.* **65**, 29–37.

DISTEL, H. (1976) Behavior and electrical brain stimulation in the green Iguana, *Iguana iguana L. Brain Behav. Evol.* **13**, 421–450.

GÓMEZ, T., RIAL, R. V., BOLAÑOS, A. and MOLINA, M. (1988) The prophylactic effect of diphenylhidantoin in the kindling effect on reptiles (*Gallotia galloti*). *Comp. biochem. Physiol.* **90C**(1), 203–205.

KARTEN, H. J. and HODOS, W. (1967) *A Stereotaxic Atlas of the Brain of the Pigeon* (*Columba livia*). Johns Hopkins Press: Baltimore, MD.

KUENZEL, W. J. and VAN TIENHOVEN, A. (1982) Nomenclature and location of avian hypothalamic nuclei and associated circumventricular organs. *J. comp. Neurol.* **206(3)**, 293–314.

LÓPEZ-GARCÍA, C., MOLOWNY, A. and PÉREZ CLAUSELL, J. (1983) Volumetric and densitometric study in the cerebral cortex and septum of a lizard (*Lacerta galloti*) using the Timm method. *Neurosci. Lett.* **40**, 13–18.

MARTÍN TRUJILLO, J. M., REGIDOR, J. and LÓPEZ GARCÍA, C. (1975) Distribution of dendritic tree types in the cerebral cortex of *Lacerta galloti*. *Expl Brain Res.* **23** (Suppl.), 135.

MOLOWNY, A., LÓPEZ, C. and MARIN, F. (1972) Estudio citoarquitectcnico de la corteza cerebral de reptiles. *Trab. Inst. Cajal Inv. Biol.* **LXIV**, 125–152.

NETCHITAILO, P., FEUILLOLEY, M., PELLETIER, G., LEBOULENGER, F., CANTIN, M., GUTKOWSKA J. and VANDRY, H. (1987) Atrial natriuretic factor-like immunoreactivity in the central nervous system of the frog. *Neuroscience* **22**(1), 341–360.

PÉREZ-BATISTA, M. A. (1976) Estudio de las áreas neurosecretorias de *Lacerta Galloti* y *Chalcides viridanus*. Licenciature Thesis, La Laguna University, Spain.

PETER, R. E. and GILL, V. E. (1975) A stereotaxic atlas and technique for forebrain nuclei of the goldfish, *Carassius auratus*. *J. comp. Neurol.* **159**, 69–102.

PETER, R. E., MACEY, M. J. and GILL, V. E. (1975) A stereotaxic atlas and technique for forebrain nuclei of the killfish, *Fundulus heterociclus*. *J. comp. Neurol.* **159**, 103–128.

PLATEL, R. (1972) Les relations ponderales encephalosomatiques chez les reptiles sauriens. *C.R. hebd. Seanc. Acad. Sci., Paris* **274**, 2181–2184.

REGIDOR, J. (1978) *Tipología Neuronal y Organización de la Corteza Cerebral de* Lacerta galloti. Publ. de la Mancomunidad de Cabildos, Plan Cultural, Las Palmas.

RIAL, R. V. and GONZÁLEZ, J. (1978) Kindling effect in the reptilian brain: Motor and electrographic manifestations. *Epilepsia* **19**, 581–589.

UMBREIT, W., BURRIS, O. and ATTANFFEN, J. (1959) *Manometric Techniques*. Burges Publ. Co.: Minneapolis.