A Stereotaxic Atlas for Diencephalic Nuclei of the Frog, 
*Rana pipiens*

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Summary. A stereotaxic apparatus was devised for frogs (*Rana pipiens pipiens*) by adaptation of a commercially available apparatus. An atlas of orienting illustrations emphasizing detailed structure and distribution of forebrain nuclei was prepared from celloidin sections and paraffin sections. Nomenclature of nuclei is discussed and an attempt made to reconcile various interpretations in the published literature.

Recently, in commencing a project which required precise penetration of electrodes or implantation of steroid pellets into the frog brain, we discovered inadequacy of detail in the single published atlas of the frog (*Rana esculenta*) brain (KEMALI and BRAITENBERG, 1969), as well as in several original papers and reviews which include drawings of parts of the brains of various frog species (ARIENS-KAPPERS et al., 1936; FRONTERA, 1952; HOFFMAN, 1963; CLAIRAMBAULT and ANTEINO, 1970; KICLITER and EBBESSON, 1976; NIEUWENHUYS and OPDAM, 1976; OKSCHE and UECK, 1976). Thus, there is a need for an anatomical atlas that will facilitate the application of stereotaxic techniques to the frog brain, particularly in studies of neuroendocrine mechanisms in a commonly studied species, the American leopard frog, *Rana pipiens*. In fact, it may be hoped that availability of such an anatomical guide may stimulate further use of *Rana pipiens* in neuroendocrine studies. We have devised an appropriate holding device for the frog, *Rana pipiens*, and we provide here the spatial coordinates that should permit finding of specific structures or nuclei in the brain for experimental purposes.

MATERIALS AND METHODS

*Animals*

Northern leopard frogs, *Rana pipiens pipiens*, collected in Ontario, Canada, and purchased from the Bay Biological Supply, Ltd., Port Credit, Ontario were used to provide measurement data for the atlas. Measurements were made in a study of ten

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adult male and female frogs of comparable size (weight ca. 50g). The usefulness and accuracy of the data provided by the atlas were tested in 5 additional frogs averaging 44g in body weight and 7.8 cm in snout-vent length.

Stereotaxic apparatus

A commercially available stereotaxic instrument for small animals (David Kopf Instruments, #900) was modified for frogs in order to hold the head immobile at three points. The ear bars of the Kopf apparatus were replaced by notched orbital bars made of plexiglass (Fig. 1, 2) that fit on the upper rim (the frontal bone) of each bony orbit. The frog mouth clamp, made of plexiglass (Fig. 1 and 2), was screwed to the median sliding plate which holds the mouth clamp of the original Kopf instrument. A frog held in this apparatus is shown in Figure 2.

The dorsal reference zero point is defined as the anterior margin of the pars impar tecti mesencephali, that is the apex of the angle formed by the dorsal midline meeting of the two optic lobes (Fig. 3). The horizontal dorsal reference zero plane is a relative one, and for each reference section it is tangent to, or touches, the highest dorsal surface at that anteroposterior level.

Preparation of frogs used for reference sections

The reference data for the atlas were based initially on a frog (body weight 50g, body length 8.2cm) which was secured in the stereotaxic apparatus. After exposure of the dorsal brain surface three steel insect pins were inserted vertically into the brain so that their tips were fixed in the cranial floor. The first pin was placed medially through the fissura sagittalis, and the other two pins were placed carefully in a transverse line 2.0mm posterior to the first pin and each 1.0mm from the midline. After fixation of the decapitated head in Bouin’s solution for 4 days, the head was cut transversely into two parts by a vertical razor blade cut parallel to the first pin. The two pieces of head were dehydrated through ethanol series and embedded in celloidin. Celloidin-blocks were cross-sectioned parallel to the cut surface at 100μm thickness, and the sections were stained with hematoxylin and eosin. Images of the stained sections were projected onto paper and traced. Every other section (i.e., 0.2mm apart) was traced in this way except for the anterior telencephalon, where
every fourth section (i.e., 0.4 mm apart) was traced. From the measurement of
distance between the reference pin holes, originally embedded 2 mm apart, a 15% shrinkage due to fixation and embedding was estimated. This value was used to estimate error in later reference coordinates.

Heads of additional 5 frogs were fixed in 10% formal saline, with or without reference pin placement and cut sagittally, or transversely along the pins for comparison, and estimation of variation. Paraffin sections of brains from 4 frogs were stained with toluidine blue and aldehyde fuchsin in order to locate Gomori-positive

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**Fig. 2.** Part of the apparatus showing a frog fixed in place (a), the orbital bar (b), and the mouth clamp (c).
neurosecretory cells in the outline drawings. In designating the position of a given brain structure, three parameters are given in millimeters. The first represents the distance anterior (+) or posterior (−) to the zero reference point; the second specifies the distance laterally right or left (R or L) from the midline; and the third represents the vertical distance below the highest surface at that anteroposterior level.

**Accuracy of the atlas**

To test the usefulness and accuracy of the data provided by the atlas, the brains of 5 frogs averaging 44 g in body weight and 7.8 cm in snout-vent length were penetrated with electrodes to positions estimated to be the anterior medial part of the nucleus preopticus (+3.4, 0.0, 3.3) and the nucleus infundibularis dorsalis (+1.2, 0.5R, 2.6). Radiofrequency lesions were made through the implanted electrodes. Brains were fixed in Bouin’s fluid, embedded in paraffin, sectioned serially at 10 μm and stained with toluidine blue. Loci of lesions in each preparation were studied with a microscope and then actual positions were traced upon copies of atlas figures and midpoints of the lesions were estimated from coordinate grids. These data are summarized in Table 1.

**Table 1.** Differences between coordinates of electrode tips placed and aimed coordinates in the nucleus preopticus and the nucleus infundibularis dorsalis

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Antero-posterior</th>
<th>Right-left</th>
<th>Vertical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinates of electrode tips placed</td>
<td>+3.69 ± 0.029*</td>
<td>0.01 L ± 0.35</td>
<td>2.94 ± 0.175</td>
</tr>
<tr>
<td>Coordinates of aimed</td>
<td>+3.40</td>
<td>0.00</td>
<td>3.30</td>
</tr>
<tr>
<td>Difference (mm)</td>
<td>0.29</td>
<td>0.10</td>
<td>0.36</td>
</tr>
<tr>
<td>Coordinates of electrode tips placed</td>
<td>+1.26 ± 0.24</td>
<td>0.09 R ± 0.037</td>
<td>2.00 ± 0.084</td>
</tr>
<tr>
<td>Coordinates of aimed</td>
<td>+1.20</td>
<td>0.50 R</td>
<td>2.60</td>
</tr>
<tr>
<td>Difference (mm)</td>
<td>0.06</td>
<td>0.41</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*a mean ± standard error  
*b numbers in the parenthesis indicate numbers of frogs used
RESULTS AND DISCUSSION

Plate Figures 1–34 constitute an atlas of locations of brain nuclei of the frog, *Rana pipiens*. Numbers above each drawing are antero-posterior positions relative to the zero point (0.0). Numbers preceded by a + indicate distance in millimeters anterior to 0.0; numbers preceded by a − indicate distance posterior to 0.0. The electrode tip must be first placed at the zero point and graduated manipulator readings should be noted at that time. To place the electrode tip at the desired position, antero-posterior distance (+ or −) between the zero point and the desired position is first noted and the electrode tip is raised slightly and moved to the proper + or − distance. Lateral settings are taken from the grids on the appropriate atlas figure and the electrode moved laterally right (R) or left (L) the indicated distance. The reference zero planes for vertical positioning passed through the highest point at any particular level of the brain and are so indicated on each figure. The electrode is then lowered to the desired position. The techniques are basically the same as developed for the goldfish by R. E. Peter and co-workers (1975).

Reliability of the atlas was checked in several ways: 1) by using it in hormone implantation and electrical stimulation experiments (Wada and Gorbman, 1977a; Wada and Gorbman, 1977b), and 2) by direct check of precision of the atlas coordinates in placement of radiofrequency lesions in specified aimed-at points (summarized in Table 1). Shrinkage in the anterior-posterior direction was not allowed for in compiling the atlas figures although this can be done easily by using the 15% shrinkage value. Accordingly, the implantations into the anterior preoptic nucleus proved to be slightly more anterior than expected. Vertical deviations in the placements into the nucleus infundibularis dorsalis were larger than expected for unknown reasons. Non-proportional differences of shrinkage of various parts of the brain is one possibility; vertical movement of the brain in a cranial cavity which it does not entirely fill is another. However, the disparities between targeted and actual stereotaxic distances were always small enough to be compatible with successful use of the atlas in lesioning experiments.

In the following sections we briefly discuss the nomenclature of the various forebrain nuclei in an attempt to reconcile the usages of previous authors. The nomenclatural usage in this atlas is basically that of Frontera (1952) and Hoffman (1963) for forebrain, and Nieuwenhuys and Opdam (1976) for midbrain.

*Telencephalon*

A recent review of the structural organization of the nonolfactory telencephalon (Kicliter and Ebbesson, 1976) has provided much of the nomenclature used for this brain area in this atlas. We provide only a brief survey of the telencephalic area.

The pallium mediale described here also has been designated in the salamander as the primordium hippocampi (Herrick, 1948) and as pallium mediale (hippocampus) in *Rana esculenta* by Kemali and Braitenberg (1969). This indicates that the nucleus was thought to be homologous with the hippocampus of mammals. We also use the more vernacular term medial pallium, according to Northcutt (1974). Vertically, the pallium mediale is separated from the septum by a thin cell-poor zone.
Dorsally, the pallium mediale gradually merges into the pallium dorsale. Accordingly, in this atlas for *R. pipiens* no separation is indicated between the pallium dorsale and the pallium mediale. The pallium laterale has been called the nucleus olfactorius dorsolateralis by Herrick (1948) or the primordium piriforme (Herrick, 1948; Hoffman, 1963) in various Amphibia. We adopted the nomenclatorial designations of Kemali and Braitenberg (1969) and Northcutt (1974). The pallium laterale is distinct, having a compact cellular region which lies along the ventricle from the pallium dorsale ventrally to the sulcus limitans lateralis. The pallium laterale, in

**Plate Figures 1–34 (p. 164–170).**

These cross-sectional drawings represent structures found at anteroposterior distances every 0.2 mm. Distances anterior or posterior to the zero point (mid-anterior margin of optic tecta) are indicated above each drawing, + indicating anterior and — posterior to the zero point. Grids given on the left halves are also drawn on a scale with 0.2 mm intervals. Black dots in 14–17 indicate approximate distribution of Gomori-positive neurosecretory neurons.

**Abbreviations.**

- **AI** amygdala, pars lateralis
- **Am** amygdala, pars medialis (Northcutt, 1974), nucleus amygdala and nucleus amygdala dorsolateralis of Herrick (1948)
- **AC** anterior commissure
- **AVA** area ventralis anterior thalami (Frontera, 1952; Kemali and Braitenberg, 1969)
- **AVL** area ventrolateralis thalami (Frontera, 1952; Kemali and Braitenberg, 1969)
- **AVM** area ventromedialis thalami (Frontera, 1952; Kemali and Braitenberg, 1969)
- **BOgl** bulbus olfactorius, glomerular layer
- **BOml** bulbus olfactorius, mitral cell layer
- **BOA** bulbus olfactorius accessorius
- **C** cerebellum
- **CGL** corpus geniculatus laterale (Frontera, 1952: Frontera divided this complex into two portions, pars ventralis and pars dorsalis), lateral geniculate body of Kemali and Braitenberg (1969)
- **ChP** choroid plexus
- **E** epiphysis
- **GC** griseum centrale rhombencephali
- **HC** habenular commissure
- **LFB** lateral forebrain bundle
- **ME** median eminence
- **MFB** medial forebrain bundle
- **NAD** nucleus anterodorsalis tegmenti mesencephali
- **NAS** nucleus accumbens septi (Herrick, 1948; Hoffman, 1963; Northcutt, 1974: This nucleus as designated by Northcutt overlapped the posterior end of the nucleus accumbens as defined by Hoffman. This structure was designated amygdala, pars medialis by Northcutt)
- **NAV** nucleus anteroventralis tegmenti mesencephali
- **NBPC** bed nucleus of the pallial commissure (Northcutt, 1974), bed nucleus of hippocampal commissure of Frontera (1952) and Hoffman (1963) (bed nucleus of the hippocampal commissure, the term used by Hoffman, was rather extensive and included amygdala as defined by other authors)
- **NCER** nucleus cerebelli
- **NDB** nucleus diagonal band of Broca (Hoffman, 1963)
- **NDLA** nucleus dorsolateralis anterior thalami (Frontera, 1952; Kemali and Braitenberg, 1969)
- **NDMA** nucleus dorsomedialis anterior thalami (Frontera, 1952; Kemali and Braitenberg, 1969)
- **NEP** nucleus entopeduncularis (Frontera, 1962). He divided this nucleus into anterior and posterior parts; Hoffman, 1963; Northcutt, 1974: This nucleus, as designated by Hoffman, included the amygdala of Northcutt)
- **NF** nucleus of the film
- **NHD** nucleus habenularis dorsalis
- **NHV** nucleus habenularis ventralis (Herrick,
1948; Hoffman, 1963; Kemali and Braithaben, 1969)

NI nucleus isthmi (Ariens-Kappers et al., 1936; Kemali and Braithaben, 1969)

NID nucleus infundibularis dorsalis, ventral lobe of pars dorsalis hypothalami of Herrick (1948), dorsal hypothalamus of Frontera (1952)

NIV nucleus infundibularis ventralis, pars ventralis hypothalami of Herrick (1948), nucleus periventricularis arcuatus and anterior part of the ventral hypothalamus of Frontera (1952)

NIP nucleus interpenduncularis (Herrick, 1948; Kemali and Braithaben, 1969)

NLS nucleus lateralis septi (Herrick, 1948; Kemali and Braithaben, 1969; Northcutt, 1974)

NMNT nucleus mesencephalicus nervi trigemini

NMS nucleus medialis septi (Herrick, 1948; Kemali and Braithaben, 1969; Northcutt, 1974)

NOA nucleus olfactorius anterior (Hoffman, 1963. He divided this nucleus into four portions: pars dorsolateralis, pars dorsomedialis, pars ventrolateralis, pars ventromedialis)

NOM nucleus of the oculomotor nerve (NIII)

NPC nucleus posterocentralis thalami (Frontera, 1952; Kemali and Braithaben, 1969)

NPD nucleus posterodorsalis tegmenti mesencephali

NPL nucleus posterolateralis thalami (Frontera, 1952; Kemali and Braithaben, 1969)

NPM nucleus profundus mesencephali

NPO nucleus preopticus (Herrick, 1948). This nucleus can be divided into anterior and posterior parts. These parts are composed, in turn, of two regions: pars parvoocularis and pars magnocellularis (Hoffman, 1963; Frontera, 1952)

NPV nucleus posteroverentralis tegmenti mesencephali

NR nucleus rotundus (Frontera, 1952; Kemali and Braithaben, 1969)

NRIS nucleus reticularis isthmi

NRS nucleus reticularis superior

NT motor nucleus of the trigeminal nerve

(NV)

NTRO nucleus of the trochlear nerve (NIV)

OC optic chiasma

OMN oculomotor nerve

ON optic nerve

OT optic tract

P parapysis

PaC pallial commissure (Northcutt, 1974), hippocampal commissure of Herrick (1948) and Hoffman (1963)

PC posterior commissure

PD pallium dorsale (Northcutt, 1974; Kemali and Braithaben, 1969), primordium pallii dorsale of Herrick (1948)

Pdis pars distalis hypophysis

PI pars intermedia hypophysis

PL pallium laterale

PLd pallium laterale, pars dorsalis

PLE pallium laterale, pars ventralis (Northcutt, 1974), pallium laterale of Kemali and Braithaben (1969), nucleus olfactorius dorsolateralis or primordium piriforme of Herrick (1948)

PM pallium mediale (Northcutt, 1974), pallium mediale (hippocampus) of Kemali and Braithaben (1969), primordium hippocampi of Herrick (1948) pars nervosa hypophysis

PN stratum griseum centrale tecti (Ariens-Kappers et al., 1936; Kemali and Braithaben, 1969)

SGC stratum griseum centrale tecti (Ariens-Kappers et al., 1936; Kemali and Braithaben, 1969)

SGP stratum griseum periventricularis tecti (Ariens-Kappers et al., 1936; Kemali and Braithaben, 1969)

SGS stratum griseum superficiale tecti (Ariens-Kappers et al., 1936; Kemali and Braithaben, 1969)

SLL sulcus limitans lateralis

STD striatum, pars dorsalis

STe striatum, pars ventralis (Hoffman, 1963; Northcutt, 1974), corpus striatum, pars dorsalis or pars ventralis of Herrick (1948)

T tegmentum

TroN trochlear nerve

TS torus semicircularis (Ariens-Kappers et al., 1936; Kemali and Braithaben, 1969)

VN vomeronasal nerve
Plate Figures 1-6.
Plate Figures 7–12.
Plate Figures 13–18.
Plate Figures 19–23.
Plate Figures 27–29.
Plate Figures 30-34.
turn, can be divided into two portions, the pars dorsalis and the pars ventralis. The neurons (perikarya) of the pars dorsalis are less compactly packed than those of the pars ventralis (Northcutt, 1974).

The septum is distinctive with distinguishable median and lateral cell masses. In this atlas they are named nucleus lateralis septi and the nucleus medialis septi after Herrick (1948) and Kemali and Braitenberg (1969). The nucleus lateralis septi lies along the cerebral ventricle and is bordered medially by the contiguous medial septum. The nucleus medialis septi is a great cell mass which extends rostro-caudally. The bed nucleus of the pallial commissure has been known also as the bed nucleus of the hippocampal commissure; however in this atlas we have employed the former name to be consistent with the usage "pallium mediale" instead of "primordial hippocampi." The bed nucleus of the hippocampal commissure referred to by Hoffman (1963) is covered by the term amygdala pars medialis here. We followed the usage of Northcutt (1974), based on the results of histochemistry of enzyme activity. The amygdala is situated in the postero-ventral part of the telencephalon, like a crescentic roof over the preoptic nucleus. Northcutt (1974) divided the nucleus into a pars medialis and pars lateralis. The pars medialis of the amygdala begins just after the nucleus accumbens separates from contact with the pars lateralis; it gradually becomes fused in the midline forming a crescent shape, and it finally disappears just anterior to the anterior commissure. The nucleus entopeduncularis appears between the pars medialis and the lateralis of the amygdala. This nucleus extends rostro-caudally along the lateral forebrain bundle. Frontera (1952) divided this nucleus into anterior and posterior parts.

**Preoptic area**

The preoptic nucleus is very distinctive in its anatomical features and the name nucleus preopticus has been commonly used by most investigators (Herrick, 1948; Frontera, 1952; Hoffman, 1963; Kemali and Braitenberg, 1969). However, the nomenclature for subdivisions of the nucleus varies among investigators. Proposed names include nucleus preopticus periventricularis magnocellularis and the nucleus preopticus periventricularis parvocellularis (Hoffman, 1963), or nucleus preopticus medialis, nucleus preopticus lateralis, nucleus periventricularis preopticus and nucleus magnocellularis preopticus (Frontera, 1952). In this atlas, we have employed the most common name, nucleus preopticus. However, it is apparent that this nucleus can be clearly divided in *R. pipiens* into two portions, pars anterior (from +3.6 to +3.0) and pars posterior (from +2.8 to +2.0). The nucleus preopticus pars anterior surrounds the preoptic recess and is separated by a thin cell-poor zone from the pars posterior. In the anterior preoptic nucleus, there are many dopaminergic neurons sending axons to the tuber cinereum (Prasado Rao and Hartwig, 1973), but no Gomori-positive neurosecretory perikarya. Functionally, the neurons in this region can concentrate sex steroids (Kelley et al., 1978) and are related to expression of sexual behavior (Wada and Gorbmam, 1977a, b). In the posterior preoptic nucleus, Gomori-positive neurosecretory perikarya are found in the ventral part of the nucleus at its anterior end and they extend upward into the posterior part. The neurons surrounding the ventricle in the preoptic nucleus are arranged in a well organized laminar pattern as Frontera (1952) and Hoffman (1963) have noted. This nucleus in frogs may be homologous to the preoptic area, nucleus supraopticus, nu-
nucleus paraventricularis, nucleus suprachiasmaticus, and anterior hypothalamus in higher vertebrates, and a variety of functions are attributed to it in various species of anurans (Hanke, 1976).

**Epithalamus**
The nucleus habenularis is clearly defined and lies just under the roof of the diencephalon (described by Herrick, 1948, in a salamander; Hoffman, 1963, in several anurans). The habenular nucleus can be divided into distinct dorsal and ventral parts. Kemali and Braitenberg (1969) refer to this nucleus in *Rana esculenta* as the nucleus habenularis; they further distinguished areas dexter and sinister, recognizing that the right and left parts of the nucleus are differentiated. We used the terms nucleus habenularis dorsalis and ventralis, without further distinctions in *Rana pipiens*.

**Thalamus**
The thalamus contains the nucleus dorsomedialis anterior thalami, nucleus dorsolateralis anterior thalami, nucleus rotundus, area ventralis anterior thalami, corpus geniculatus laterale, area ventrolateralis thalami, area ventromedialis thalami, nucleus posterocentralis thalami, nucleus posterolateralis thalami. The area ventralis anterior thalami extends from just posterior to the anterior commissure. This nucleus gradually diminishes posteriorly, and the area ventromedialis thalami and the area ventrolateralis thalami appear. The dorsally situated nucleus dorsomedialis anterior thalami and the nucleus dorsolateralis thalami are replaced or followed posteriorly by the nucleus posterocentralis thalami and the nucleus posterolateralis thalami. The elongated nucleus rotundus is a distinct landmark bordering the area ventrolateralis thalami and the nucleus posterocentralis thalami. The nucleus posterolateralis thalami and the area ventrolateralis thalami are situated still more posteriorly in the dorsal thalamus. The corpus geniculatus laterale is a thin dorsoventral extensive strip bordering the lateral thalamus.

**Hypothalamus**
The nucleus infundibularis dorsalis and the nucleus infundibularis ventralis were designated as the dorsal hypothalamus and the ventral hypothalamus (+ nucleus periventricularis arcuatus), respectively, by Frontera (1952). However, we employed the nomenclature of Peute (1973) and Chacko et al. (1974) who found monoamine fluorescence in the nucleus infundibularis dorsalis and the paraventricular organ which separates these two nuclei. The nucleus infundibularis ventralis may be the homologue of the nucleus arcuatus in mammals, but it would be prudent to avoid this term before establishment of its function.

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