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Mensaje editorial:

Este número de Archivos Mexicanos de Anatomía que corresponde al VII año de nuestra publicación, marca la etapa de la Directiva 1966-1969.

Contiene algunos importantes trabajos que se presentaron en el I Congreso Panamericano y III Nacional de Anatomía.

De la motivación de la Directiva y de la actuación de todos los integrantes de la Sociedad Mexicana de Anatomía, dependerá el alcanzar elevadas metas científicas.

Tres etapas ha compaginado nuestra Sociedad: la primera, de organización y consolidación, que culminó con la realización del I Congreso Nacional; la segunda, de difusión nacional que finalizó con el brillante II Congreso efectuado en San Luis Potosí, y la tercera, de proyección internacional en que se anotó un éxito extraordinario en el I Congreso Panamericano y III Nacional realizado en la Ciudad de México.

Las Directivas correspondientes y el entusiasmo de los anatomistas nacionales y extranjeros, han sido decisivos para llegar a obtener el mayor relieve a todas estas etapas históricas.

Ahora toca a la Directiva 1966-1969 forjar la cuarta etapa de reestructuración, para acrecentar las filas y reintegrar en forma definitiva la vida de nuestra Sociedad.

La Directiva actual y el Consejo Editorial, esperan seguir contando con el mismo interés y entusiasmo de todos los integrantes de la Sociedad Mexicana de Anatomía, para llegar a vencer los obstáculos que se presenten y hacer una realidad el programa que se han trazado.

Profr. y Dr. Liberato J. A. DiDio

Señor Presidente

Autoridades aquí presentes,

Señores y Señoras.

En lugar de suplicarles que me perdonen por querer hablar castellano, voy a contarles una anécdota: Había en Estados Unidos un americano que enseñaba japonés y que fue a hacer una gira en Japón con un grupo de sus alumnos. Después de la visita al País, el Gobierno japonés les ofreció un banquete. Naturalmente los alumnos pidieron al Profesor que hablase en japonés para agradecer las atenciones recibidas. El Profesor hizo un vibrante discurso, y al momento de la Clausura, el Jefe del Gobierno Japonés dijo: "ahora me puedo dar cuenta de la necesidad de estrechar cada vez más la amistad entre Japón y Estados Unidos, pues aunque no haya entendido al orador, puedo notar que algunas palabras de los dos idiomas se parecen mucho."

Espero que al finalizar mis palabras, el resultado sea un poco diferente. Por otro lado, voy a aprovechar esta oportunidad para hablar en castellano como una demostración de mi satisfacción y orgullo por haber sido nombrado mexicano del Sur, al terminar mi conferencia sobre "El órgano Subcomisural" en el Hospital Nacional de Neurología.

De inmediato quiero recordarles, que amigos son todos aquellos que interpretan nuestros defectos como cualidades, mientras que los enemigos transforman

nuestras virtudes en vicios. Por todas las demostraciones de afecto que he recibido de los mexicanos, puedo decir que ellos son mis mejores amigos, y que si pudiera, aquí me quedaría para siempre.

Solamente la infinita generosidad de los mexicanos, puede justificar la selección de mi nombre para dirigirles la palabra en la Sesión de Clausura.

En esto, yo veo inmediatamente una ventaja: como soy el peor orador entre los anatomistas, ustedes pueden concluir **ipso facto**, que cualquier otro sería mejor.

Pero en las presentes circunstancias yo estoy seguro que la invitación que me fue hecha por el ilustre señor Presidente doctor Fernando Quiroz Pavía, fue un homenaje a la Anatomía de los Estados Unidos de Norte-América, particularmente a la "American Association of Anatomists", que tengo el honor y el placer de representar oficialmente en este Primer Congreso Panamericano de Anatomía, y para la fundación de la Asociación Panamericana de Anatomía.

La realización de este excelente Congreso y la fundación de la Asociación Panamericana de Anatomía, constituyen la materialización de un sueño. Seguramente todos ustedes tuvieron la oportunidad de leer el Acta del Congreso de la "American Association of Anatomists", publicada en "The Anatomical Record" (140: 238, 1961), en la cual sugerimos la organización de una Asociación Panamericana. Creo que para el registro histórico debemos mencionar, que el eminente señor Dr. H. Stanley Bennett, en la misma

Sesión de negocios, propuso que el Comité Ejecutivo de la Asociación de Anatomistas Americanos, estudiase la manera más conveniente de alcanzar nuestro objetivo. De este estudio resultó el nombramiento del señor Dr. Donald Duncan para presidir una comisión encargada de apoyar firmemente la iniciativa que, a esta altura, los mexicanos habían tomado. Así, en el último Congreso de la "American Association of Anatomists" realizado en abril en San Francisco, el antiguo Presidente, señor Dr. Donald W. Fawcett y el nuevo Presidente señor Dr. Donald Duncan, me informaron que había sido nombrado Delegado Oficial para los eventos científicos y sociales a realizarse en esta Ciudad de México, y que mi gran amigo señor Dr. Larry F. Cavazos concurriría como suplente.

Por esto, para el Dr. Bennett, el Dr. Duncan y todos los participantes norteamericanos y canadienses, cuyos nombres son muy numerosos para leerlos en este momento, suplico a ustedes un especial aplauso. La organización y realización de los Congresos Panamericano y Mexicano y la fundación de la Asociación son tareas que solamente por milagro podrían acontecer. Este milagro, fue realizado por los anatomistas mexicanos de una manera maravillosa; por esto, yo también ahora puedo recitar con nuestros amigos mexicanos, el **Credo** del poeta contemporáneo Ricardo López Méndez:

...México, creo en ti,
en tus cosechas de milagrería,
que sólo son deseo en las palabras.
Te contagias de auroras que te cantan,
¡Y todo el bosque se te vuelve carne,
y todo el hombre se te vuelve selva!...

Necesitaría un periodo de tiempo mucho más largo del que tengo a mi disposición, para poder agradecer en nombre de todos a cada uno de los mexicanos que nos han atendido y que consiguieron realizar con tanta perfección los Congresos que hoy se clausuran.

La omisión de sus nombres no significa disminución del calor de nuestra gra-

titud; sin embargo, es mi deber nombrar por lo menos a dos mexicanos ilustres: los señores Doctores y Profesores Quiroz Gutiérrez y Quiroz Pavía. El Prof. Quiroz Gutiérrez, decano de la Escuela de Medicina, Jefe de la Escuela Mexicana de Anatomistas, tradicional inspirador y orientador de los más importantes eventos científicos de México, respetado con veneración por colegas, antiguos alumnos y estudiantes, constituye el símbolo vivo de la Anatomía Mexicana. Yo vine a tener el honor de conocerlo aquí al inicio del Congreso, y puedo verificar que en muchos aspectos, entre los cuales el de caracterizarse como un apóstol de la Anatomía, me recuerda a mi maestro el señor Doctor y Prof. Renato Locchi, también él, una de las figuras preponderantes de la Anatomía Latino-americana. Dios no quiso que el Prof. Quiroz Gutiérrez participara con nosotros en todas las actividades del Congreso, pero estoy seguro que en Su suprema omnipotencia está oyendo las oraciones de todos sus familiares, amigos y Congresistas que hacen votos para un pronto restablecimiento y para que esté presente en el próximo Congreso. Para el Prof. Quiroz Gutiérrez les sugiero un vibrante aplauso.

El Prof. Quiroz Pavía, ilustre Presidente, continúa el camino trazado por su progenitor, acompañado por una legión de dedicados y entusiastas anatomistas, que honran distinguidamente a su País. No sabemos cuál de sus cualidades debemos admirar más; si el dinamismo de sus actividades; el calor de sus expresiones de amistad o la sinceridad de sus actitudes. El resultado final de estos eventos, son la comprobación evidente de mis afirmaciones.

La Asociación Panamericana de Anatomía, es hoy una realidad palpable. Todos los anatomistas del continente Americano, sin barreras geográficas, están unidos bajo el espíritu único de hacer progresar nuestra ciencia, animados por un deseo de cooperación igualitaria y por ansias de integración sin ninguna depen-

dencia. Los Anatomistas Americanos hoy no se restringuen más a los de Estados Unidos; hoy incluyen a todos los que se dedican al estudio de la Anatomía, desde la Patagonia hasta Alaska. Hablaremos nuestras propias lenguas y para mejor entendernos utilizaremos la misma nomenclatura anatómica. Pero estamos seguros que nos entenderemos mejor con el afecto y el calor de nuestras relaciones de amistad que se manifestaron tan elocuentemente en este Primer Congreso.

Naturalmente, que como consecuencia de nuestro intercambio, habrá los que pueden ayudar y los que deberán ser ayudados. El nivel de nuestras relaciones no tendrá ningún carácter de favor sino de honor, el honor de poder auxiliar sin esperar por compensación ni gratitud; en pocas palabras, será la voluntad de ayudar por el placer de hacerlo. Como no he tenido la oportunidad de participar en el programa Social, por obvias razones, voy a basarme en la opinión de mi esposa. Por sus palabras, puedo declarar, que las mujeres mexicanas están entre las más guapas y elegantes anfitrionas que hemos conocido en Congresos Internacionales. A las encantadoras señoras y señoritas mexicanas, expreso la admiración y el agradecimiento de todos los Congresistas. Esta misma manifestación de gratitud se extiende por nuestro intermedio a todos los mexicanos que organizaron el

Congreso, desde la Presidencia hasta los miembros del personal administrativo, que, con su espíritu de colaboración y cortesía, nos hicieron sentirnos como si estuviéramos en nuestra propia casa.

Es imposible expresar todo lo que siento, con mi pobre vocabulario y para mejor llegar al corazón de los mexicanos voy a leer unos versos de alguien que habló con la verdadera alma mexicana y que expresa mucho de lo que desearía decirles:

Son las estrofas finales de Suave Patria, compuestas por el consagrado poeta Ramón López Velarde.

...Trueno de nuestras nubes, que nos
[baña
de locura, enloquece a la montaña,
requiebra a la mujer, sana al lunático,
incorpora a los muertos, pide el Viá-
[tico,
y al fin derrumba las madererías
de Dios, sobre las tierras labrantías.

Trueno del temporal: oigo en tus
[quejas,
crujir los esqueletos en parejas:
oigo lo que fue se fue, lo que aún no
toco, y la hora actual con su vientre de
coco.

Y oigo en el brinco de tu ida y venida,
oh trueno, la ruleta de mi vida.
Muchas y muchas gracias

Actividad de Acetilcolinesterasa en cerebros de ratas con anoxia

Por José Morales L., M. D. Profesor de Anatomía de la Facultad de Medicina de la Universidad Mayor de "San Andrés" La Paz, Bolivia.

Summary: By the histoquimic method of Koelle and Friedenwald we are investigating the acetilcolinesterasa activity in the Pentral Venc System of albin rats submitted to an anoxie, acute, chronic process. The sections are practiced in a criostat 15° C. The tissue is not fix so it may keep intact the enzyme activity. There are demonstrated exceptions to the general localization rule of the enzymatic reaction, upon the gray substance and the white substance. The A.C.E. activity is independent of the nervous funtional component. This is proved because this activity is positive in motor nuclei sensorial signification and in integrative areas of Pentral Venc System.

Resumen:—Por el método histoquímico de Koelle y Friedenwald se investiga la actividad de acetilcolinesterasa en el sis-

tema nervioso central de ratas albinas sometidas a un proceso de anoxia aguda y crónica. Las secciones se practican en un criostato a —15° C. El tejido se procesa sin fijación alguna con el objeto de conservar intacta la actividad de la enzima. Quedan demostradas excepciones a la regla de localización general de la reacción enzimática sobre las sustancias gris y blanca. Que la actividad de A.C.E. es independiente del componente funcional nervioso, se comprueba por el hecho de ser positiva en núcleos motores, estructuras de significación sensorial y en áreas integrativas del S.N.C.*

Los trabajos de R. Debijadji y asociados y otros autores anteriores a éstos, han permitido evidenciar cambios significativos en la actividad monoaminérgica en el Sistema Nervioso Central de animales sometidos a hipoxia hipóxica (8). Estas

* Los resultados preliminares del presente estudio fueron dados a conocer en el VII Congreso Colombiano de Patología efectuado en la Ciudad de Cali, en noviembre de 1964.

modificaciones, según parece, son el efecto del aumento en la producción y liberación por parte del tejido nervioso, de monoaminas y catecolaminas consecutivamente a la disminución de la tensión parcial de oxígeno en la sangre que llega al cerebro. El mecanismo bioquímico de tales hallazgos y los circuitos nerviosos comprometidos, no han sido interpretados adecuadamente hasta el presente.

En contraste con los estudios realizados sobre los aspectos anteriores, poco o nada se encuentra en las fuentes usuales de información acerca de la actividad de la acetilcolinesterasa en condiciones similares de experimentación. En este trabajo pretendemos investigar, utilizando un procedimiento histoquímico, las posibles alteraciones de la actividad de A.C.E. en cerebros de ratas que fueron sometidas a un proceso de anoxia anóxica, tanto crónica así como aguda.

La A.C.E. es una enzima que se encuentra especialmente en las terminaciones nerviosas llamadas colinérgicas; sin embargo, ésta no es su única y exclusiva localización. Ha sido estudiada extensamente de preferencia a nivel de placa motora, estructura en la cual, ofrece una actividad notable (5, 9, 10). De acuerdo con Nachmansohn, D. (22), en una sola placa motora se hidrolizan 1.6×10^{-9} moléculas $\therefore 0.2 \times 10^{-6}$ ug de acetilcolina por milisegundo.

Los estudios de Dale, Loewi y varios otros (6, 7, 11, 12, 13, 15, 24), han llegado a establecer que la acetilcolina, ac-

túa a nivel sináptico como un agente neuro-humoral que sustituye la corriente eléctrica en los fenómenos de transmisión y propagación del impulso nervioso. Koelle y Friedenwald (18), comprobaron que ciertas zonas del sistema nervioso de los mamíferos, ofrecen mayor actividad de A.C.E. que otras. Los hallazgos de estos investigadores fueron confirmados y ampliados en estudios histoquímicos y bioquímicos posteriores, con lo cual ha sido posible un conocimiento más completo de la distribución, localización e intensidad de actividad de la enzima en cuestión, a través de los tejidos y las diferentes estructuras celulares (2, 12, 14, 17).

La Caracterización química de las variedades de colinesterasas constituye un problema no bien solucionado en la actualidad. Frecuentemente se utiliza y acepta la agrupación de estas enzimas en dos tipos principales (Cuadro I). Para los efectos de esta clasificación se tiene en cuenta una serie de factores y propiedades de tales fermentos, como ser: — Sus cualidades histoquímicas, sus características fisiológicas, la capacidad específica de hidrólisis sobre determinados sustratos, su localización en los diversos tejidos y en las distintas partes de la célula, etc. (3, 6, 12, 14, 25).

En el cuadro siguiente se hace la comparación entre algunos de los principales rasgos propios de ambos tipos de colinesterasas.

CUADRO I

Acetilcolinesterasa

Otras colinesterasas

DISTRIBUCION

Placa motora
Eritrocitos
Ganglios simpáticos
S. N. C. sustancia gris

Hígado
Plasma sanguíneo
Mucosa intestinal
S. N. C. sustancia blanca

ESPECIFICIDAD SOBRE SUSTRATOS

Acetilcolina
Propionilcolina
Butirilcolina

Butirilcolina
Propionilcolina
Acetilcolina

NUMERO DE SITIOS ANIONICOS

Uno o dos
(Tejidos eléctricos)

Cero a uno
(Plasma humano)

CURVA DE ACTIVIDAD CON ACETILCOLINA COMO SUSTRATO

En forma de campana. (Inhibición por exceso de sustrato)

En forma de S. (No hay inhibición por exceso de sustrato)

NOMENCLATURA

Colinesterasa verdadera
Colinesterasa tipo e
Colinesterasa específica
C. E. I.

Pseudocolinesterasa
Colinesterasa tipo s
Colinesterasa inespecífica
C. E. II

Material y Método:

Ratas albinas adultas y normales, en número de ocho, fueron introducidas en una cámara parcialmente cerrada y en cuyo interior permanecieron por un tiempo que varió entre siete y veinte días, con alimentación corriente. Interdiariamente se toman muestras del aire del interior del recipiente, las cuales son analizadas pro-

tamente, obteniéndose el siguiente promedio en volúmenes por ciento:

CO ₂	2.502
O ₂	16.632
N ₂	80.638

Normalmente las concentraciones volúmetricas proporcionales de estos gases en el laboratorio son las siguientes: CO₂ 0.04, O₂ 20.64, N₂ 79.19.*

* Los trabajos se realizaron en los laboratorios de Biología e Histoquímica de la Universidad del Valle, Cali-Colombia.

A otras cuatro ratas se les hizo inhalar CO_2 en forma masiva, hasta que resultaron en coma. Los animalitos son muertos por decapitación y se extrae con cuidado el S.N.C.; enjuágaselo rápidamente en agua fría con el objeto de eliminar la sangre que pudo haber quedado adherida a la superficie encefálica. Mediante cortes coronales y transversales obtiéndose trozos de 4 a 8 mm de espesor, los cuales son introducidos de inmediato en un criostato Harris N° M 40, enfriado a -15°C . La operación completa tiene una duración promedio de 4 minutos. Una vez congelado el tejido se practican secciones coronales y transversales de 8 micrones de espesor, las cuales son colectadas en portaobjetos y transportadas luego hacia el medio de incubación. El procesamiento ulterior se realiza de conformidad al método de Koelle y Friedenwald (18, 20, 21), en el que se hace actuar la acetil-tio-colina como sustrato.

Sirvió de control o "blanco" el S.N.C. de cuatro animalitos de la misma especie, los cuales no fueron sometidos al proceso anóxico. También se elaboran controles positivos con músculos diafragma y psoas, y controles negativos, esto es, sin sustrato. Algunas secciones son coloreadas con hematoxilina-eosina y otras son tratadas con solución acuosa de eserina al $3 \times 10^{-5} \text{ M}$ (inhibidor de la enzima) por espacio de 30 minutos, previamente a la introducción al medio de incubación. Ocasionalmente algunos cortes son coloreados con toluidina azul o en safranina O para alcanzar mayor contraste. La incubación tiene lugar a 37°C para espacio de 60 minutos. De acuerdo con las recomendaciones de Sabatini, D. D., y asociados (23), no usamos ningún fijador con el propósito de preservar la actividad enzimática.

Resultados.

Se observa al microscopio un total de 640 cortes procesados para la reacción de A.C.E. De estos cortes, 150 corresponden

a las ratas controles o "blanco". Por otra parte, 120 secciones distintas a las anteriores, se utilizaron para los diferentes controles, positivo, negativo y con inhibidor de la enzima.

En general, la reacción se presentó en estructuras de sustancia gris situadas profundamente en el S.N.C., como apreciamos en las fotografías que ilustran el presente trabajo. La corteza cerebral y la sustancia blanca no ofrecieron relación histoquímica. Esta reacción estuvo también ausente en las regiones hipotalámicas; no obstante, otros autores (1, 19), afirman haber hallado actividad de colinesterasa en dichas estructuras, aunque con métodos diferentes al desarrollado aquí.

Los controles positivos de músculos mostraron invariablemente fuerte actividad de A.C.E. en el sitio correspondiente a las placas motoras (figura 1). En los controles negativos y las secciones tratadas con eserina, no se evidencia reacción alguna.

El Cuadro II que insertamos a continuación, nos da una idea de ubicación e intensidad de la reacción enzimática según las zonas del sistema nervioso de la rata y además, nos permite resumir nuestros principales hallazgos.

CUADRO II

Reacción histoquímica intensa

Placa motora (control positivo)
 Neoestriatum núcleo caudado y putamen
 Colículo superior
 Núcleo interpeduncular
 Sustancia nigra
 Núcleos sensorial principal y motor del V par
 Núcleo del facial
 Núcleo del hipogloso
 Asta anterior de la médula espinal (cervical)

Reacción histoquímica débil

Tálamo: núcleos anteriores y laterales

Cerebelo: capa granular

Hipocampo: capa 2 del girus dentado y lámina de las pirámides del cuerno de Ammón

Núcleo del III par

Núcleo dorsal motor del vago

Asta posterior de la médula espinal

Discusión.

El neostriatum (figuras 2 y 3), ofrece una perfecta diferenciación de sus componentes grises y blancos. Sobre la sustancia blanca, la cual está constituida de fibras nerviosas mielinizadas, no se produjo depósito de sulfuro de cobre, producto final de la reacción. A nivel de la sustancia gris de estos núcleos basales no fue posible distinguir los contornos celulares, no obstante que la actividad enzimática en este sitio, se manifiesta muy claramente. La distribución del sulfuro de cobre sobre la parte celular del núcleo caudado (figura 2) y la respectiva del putamen (figura 3), se presenta en forma característica permitiendo la diferenciación perfecta de sus partes neuronales y axonales.

Los colículos superiores mostraron una reacción enérgica en sus capas superficiales, las cuales son ricas en cuerpos celulares (figura 4). La intensidad de la reacción tiende a disminuir hacia los estratos profundos. Aquí tampoco fue posible discernir contornos celulares ni apreciar la estratificación tan característica de estas estructuras nerviosas en otras clases de coloraciones. El núcleo interpeduncular es fuertemente positivo (figura 5), lo mismo que las porciones reticular y compacta de la sustancia nigra (figura 6).

La reacción enzimática adquirió caracteres notables por su intensidad y la nitidez con que permitió la apreciación de los contornos de las células, en los núcleos: Sensorial principal del V par craneal, motor del mismo nervio, del facial (figura 7), hipogloso (figura 8) y parte

medial del asta anterior de la médula espinal en sus segmentos cervicales superiores. La visualización de los límites celulares se efectúa sin ninguna dificultad en todas estas formaciones aun sin el empleo de coloración de contraste. A mayor aumento las neuronas de tales núcleos ofrecen una delgada franja de color negro la cual rodea más o menos completamente el pericardio y la iniciación de algunas prolongaciones neuronales, tratando de reproducir la disposición de las terminaciones nerviosas sobre la membrana del cuerpo y dendritas de la célula nerviosa (figura 9).

El menor depósito de sulfuro de cobre en los núcleos talámicos, el cerebelo, hipocampo, etc. (Cuadro II), nos induce a pensar que la actividad de A.C.E. es manifiestamente menor en tales regiones, antes que interpretar estas observaciones como el resultado de variaciones en el método de investigación. En el cerebelo la capacidad hidrolítica sobre el sustrato específico es mayor en la capa granular (figura 10), lo cual estaría de acuerdo con los estudios de Austin y coautores (4).

Buen número de placas histológicas examinadas presentaron cristales de tiocolato de cobre en cantidades variables, así como también la difusión de la reacción enzimática hacia regiones que normalmente no tienen este tipo de actividad. Estos artificios del procedimiento técnico podrían ser evitados según Koelle (16) mediante ciertas modificaciones de su método original.

Conclusiones.

Después del examen detenido y minucioso de los diferentes sectores del S.N.C. de la rata, nosotros no fuimos capaces de observar cambios significantes, ni de intensidad ni de localización de la reacción histoquímica entre los tejidos pertenecientes a las ratas con anoxia y los controles. Empero, con el objeto de complementar este trabajo y precisar mejor los

resultados experimentales, hemos decidido realizar cuantificaciones bioquímicas de la actividad de A.C.E. y de colinacetylase en condiciones similares a las adoptadas aquí.

Si bien en términos generales y tal como quedó establecido en el Cuadro I, la sustancia blanca carece de este tipo de actividad enzimática, mientras que la sustancia gris es uno de los puntos donde ordinariamente se la encuentra, nuestro actual estudio permite evidenciar la existencia de varias excepciones a esta regla, pues no toda la sustancia gris es reactiva (corteza, núcleos hipotalámicos, bulbo olfatorio, paleoestriatum, etc.), ni toda la sustancia blanca permanece inactiva durante el proceso histoquímico (las raíces del nervio facial son un buen ejemplo de este aserto, figura 7).

La distribución de la reacción de A.C.E. no guarda relación con los componentes funcionales del sistema nervioso central de la rata, pues se la encuentra en estructuras cuya significación funcional es desigual, como en el caso de los núcleos motrices del V, VII y XII pares craneales, del

núcleo sensorial principal del V par y el asta posterior de la médula espinal; o de los núcleos talámicos y basales mencionados más arriba, los cuales son centros de integración de la actividad nerviosa.

No podemos concluir fundándonos en estos hallazgos sobre la ausencia de localizaciones exclusivas de actividad de A.C.E. o de catecolaminas en el S.N.C., hipótesis que es apoyada por varios autores (1, 19, 22).

La disminución del aporte de oxígeno y el incremento del CO₂ en la intimidad del tejido nervioso, producen cambios en la actividad monoaminérgica tal como se dijo anteriormente (8). Ahora bien, teniendo en cuenta que las monoaminas y catecolaminas son sustancias transmisoras o mediadoras del impulso nervioso a nivel sináptico; es muy posible que bajo condiciones semejantes, la actividad colinérgica tendría que ofrecer algún cambio en alguna dirección definida. No obstante, nuestras interpretaciones sobre este aspecto, son por el momento, puramente especulativas.

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FIG. Nº 1

Músculo Diafragma. Intenso depósito de sulfuro de cobre a nivel de las placas motoras (P). Visualización de los núcleos celulares debido a la coloración de contraste. Obsérvese el filete nervioso (N) dividiéndose en dos ramas las cuales alcanzan las respectivas placas motoras. Cada fibra muscular (F) cuenta con una placa motriz 3.5 X. Rata control.

FIG. Nº 2

Neostriatum. Cabeza del Núcleo Caudado. Apreciable actividad en sus porciones medial y basal. Ninguna respuesta en la Comisura Anterior (C A), la cual está constituida de axones mielinizados. 3.5 X. Rata con anoxia.

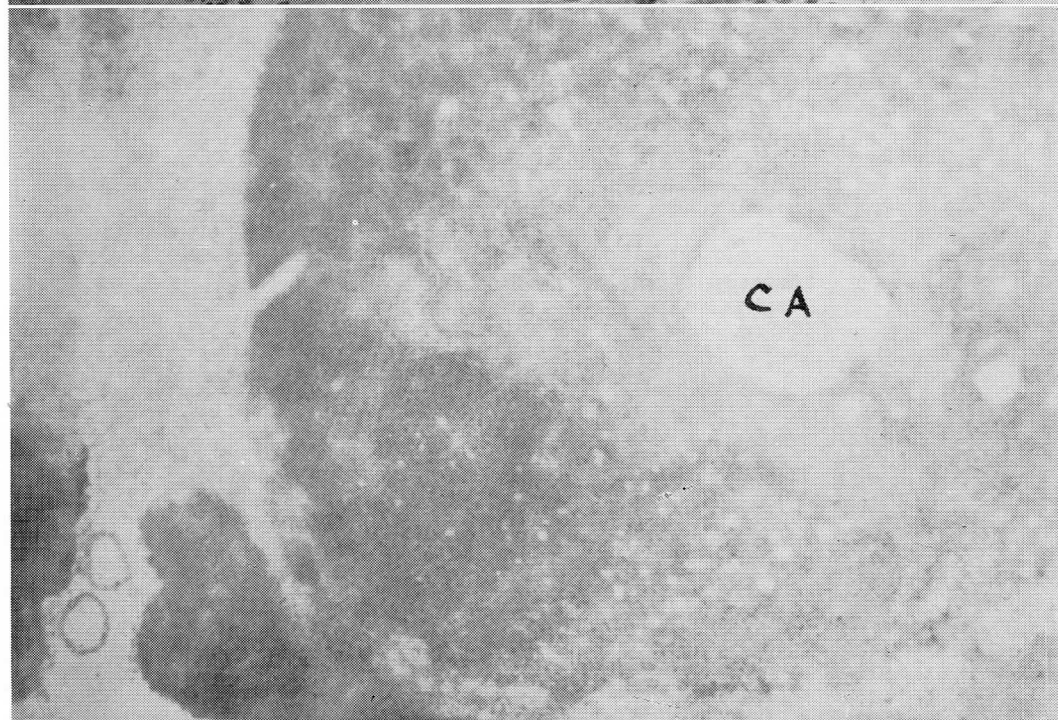
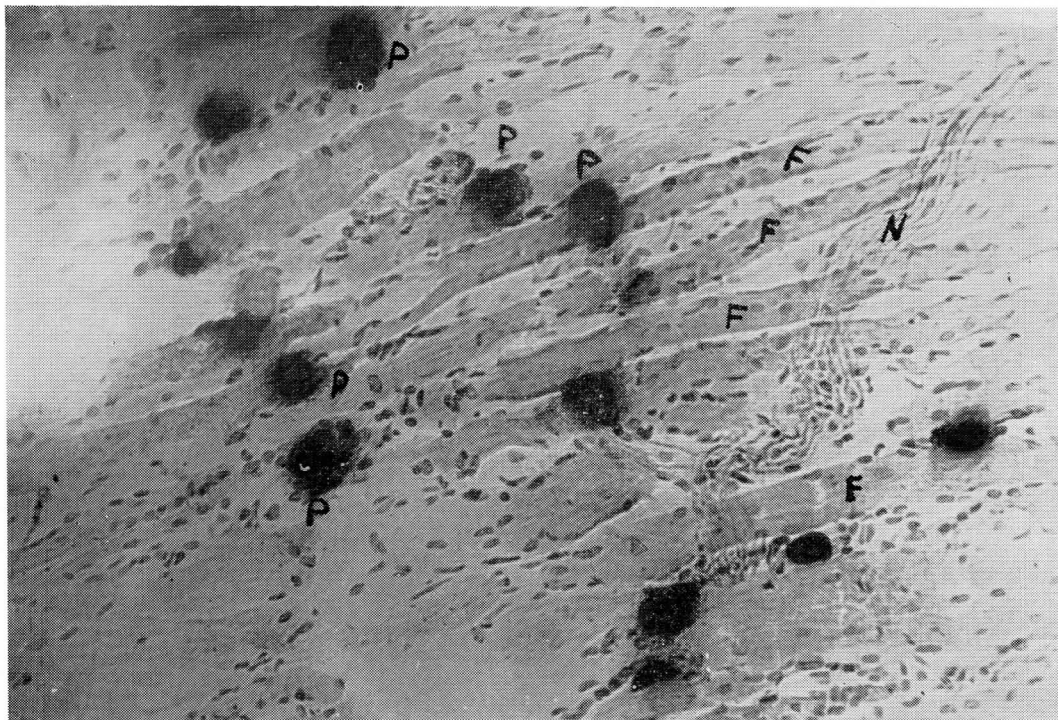


FIG. N° 3

Neostriatum. Putamen. La diferenciación entre las estructuras blancas y grises se aprecia con toda nitidez, dándole su aspecto estriado característico. Las áreas celulares son fuertemente reactivas y aparecen de color negro. El Hipocampo (H) y una zona vascular del Paleostriatum (P E), ofrecen discreta actividad. Obsérvese el ventrículo lateral con el plexo coroideo (V L). 3.5 X. Rata con anoxia.

FIG. N° 4

Colículo Superior. Las capas superficiales se muestran más activas que las profundas. La Corteza Cerebral (C) no presenta depósito de sulfuro de cobre, F. I. fisura interhemisférica. 3.5 X. Rata con anoxia.

FIG. N° 5

Núcleo Interpeduncular. El producto final de la reacción histoquímica se deposita principalmente a nivel de su parte dorsal. Sus porciones laterales son menos reactivas, mientras que la zona central es débilmente positiva. 3.5 X. Rata con anoxia.

FIG. N° 6

Sustancia Nigra. A través de esta estructura se deslizan las raíces del tercer par craneal (flechas). Nótese la débil reacción en el Núcleo del Oculomotor (N O). El Núcleo Interpeduncular es visible en las partes inferior y media de la figura, como una zona irregularmente circular. 3.5 X. Rata con anoxia.

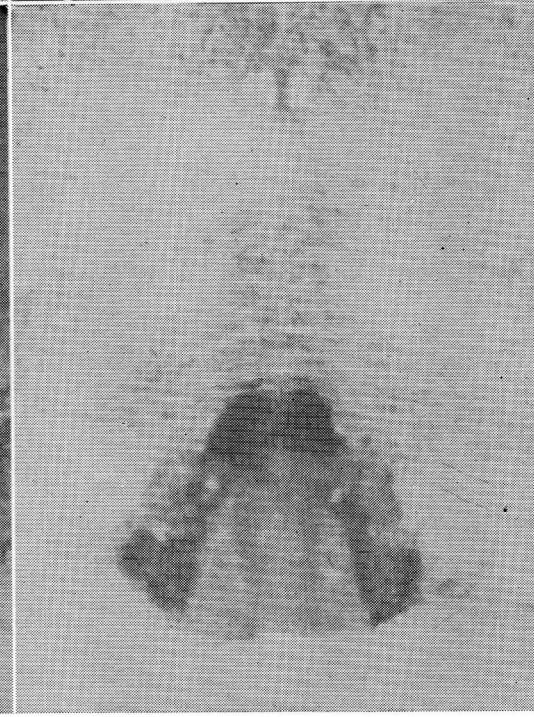
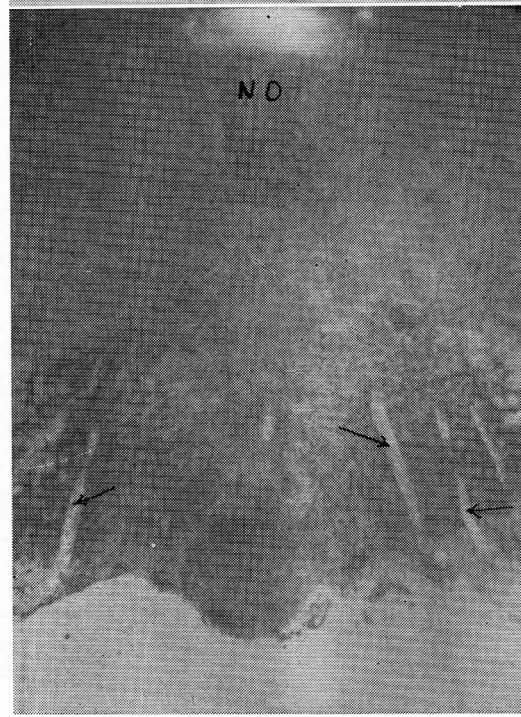
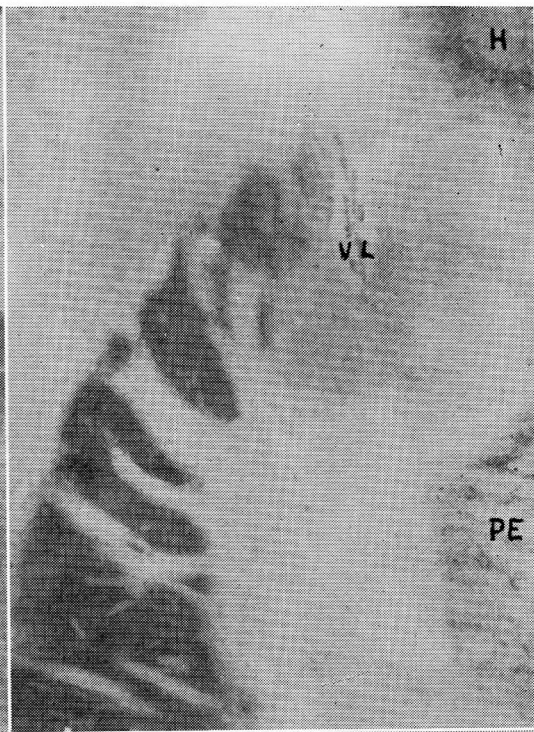
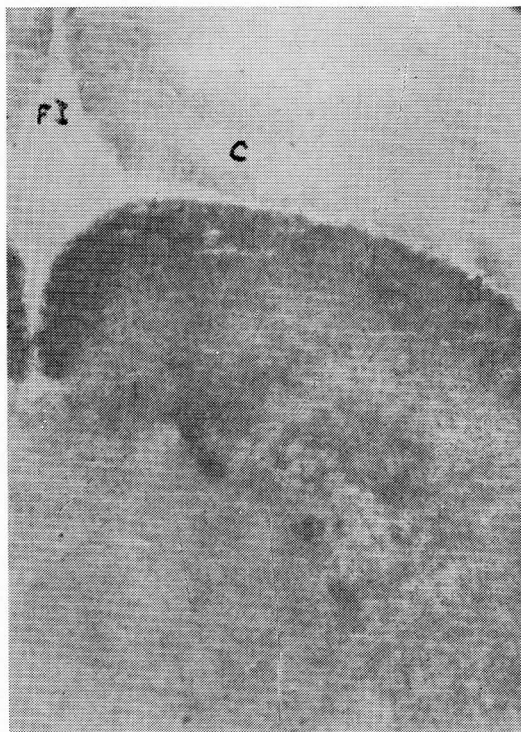


FIG. Nº 10

Neuronas del Núcleo Motor del Facial. Obsérvese la intensa reacción en la periferia del cuerpo y de una dentrita de dos neuronas contiguas. 45 X. Rata control.

FIG. Nº 8

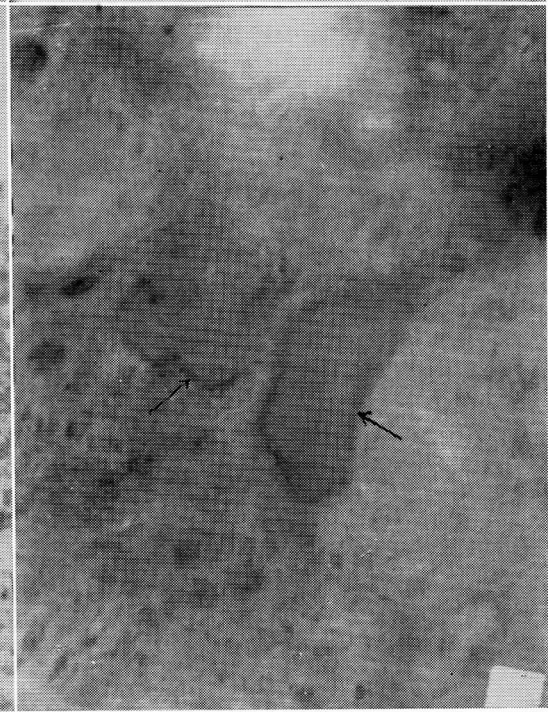
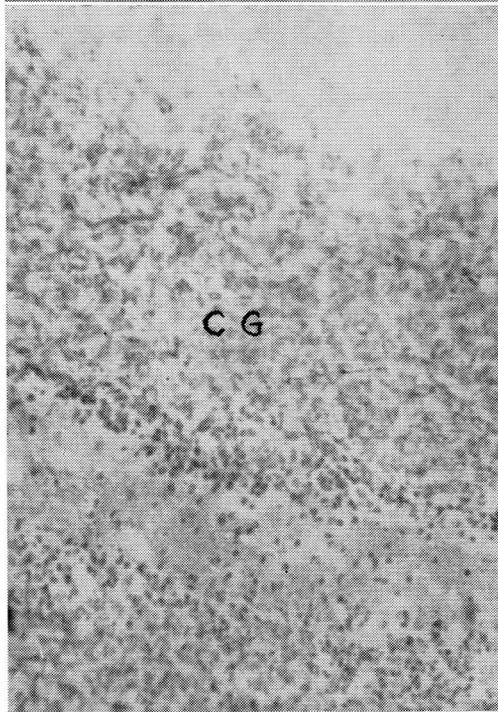
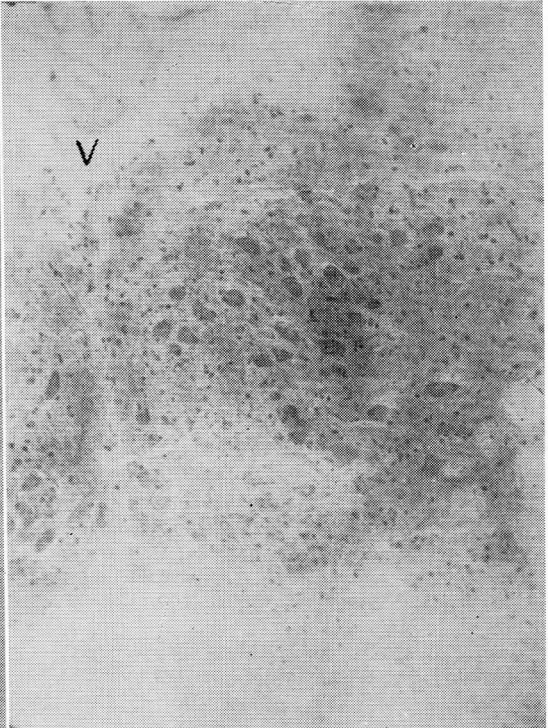
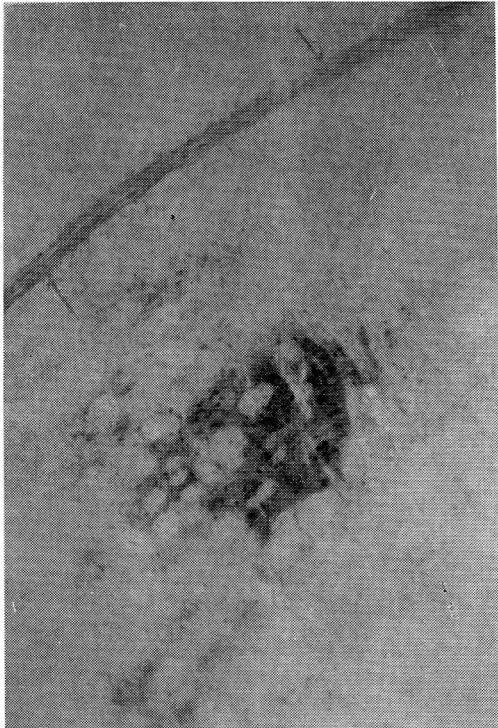
Núcleo del Hipogloso. Correcta delimitación de los contornos neuronales. V. cuarto ventrículo con el respectivo plexo coroideo. 10 X. Rata con anoxia.

FIG. Nº 9

Cerebelo. Reacción evidente, aunque débil en la capa granular (C G), de la corteza cerebelosa. 10 X. Rata control.

FIG. Nº 7

Núcleo Motor del VII Par. Abundante depósito de sulfuro de cobre a nivel del Núcleo del Facial. Las raíces de este nervio son positivas a la reacción histoquímica mientras que las del Oculomotor se ofrecen negativas, conforme se aprecia en la figura anterior (flechas). 3.5 X. Rata control.



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An integrated approach to the teaching of biological structure in the first year medical curriculum

by

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R E S U M E N

Con la iniciación de la instrucción en la nueva Escuela de Medicina de la Universidad de Nuevo México en 1964, se programaron los aspectos estructurales de medicina biológica, como una presentación interdisciplinaria.

Durante las primeras ocho semanas, se presentan conceptos básicos de Anatomía, por medio de disección de las extremidades. Al mismo tiempo, se estudia la anatomía microscópica de las células y de los tejidos fundamentales en estrecha asociación con la presentación de los principios bioquímicos.

Como continuación a la introducción de estos conceptos, se dedica un período de dos semanas al estudio de los glóbulos rojos. Se considera la glicolisis, hemopoyesis, síntesis de la hemoglobina y de las influencias metabólicas de selección de las funciones de los glóbulos rojos. Se presentan brevemente los órganos linfáticos en relación con los fenómenos inmunológicos, al mismo tiempo con una exposición microbiológica

durante tres semanas. Una breve consideración de las propiedades de contracción y conducción de los músculos y los nervios respectivamente, precede a la iniciación de la biología integral de órganos y sistemas.

La biología cardio-vascular-pulmonar, forma un conjunto interdisciplinario de enseñanza durante seis semanas, seguido de dos semanas de biología renal, tres semanas de biología gastro-intestinal y cuatro semanas de endocrinología. En todas estas áreas que sirven de tema, se representan a simple vista y al microscopio aspectos anatómicos ultraestructurales y de desarrollo como componentes integrales con las presentaciones de la fisiología, biología, y farmacología.

Durante tres semanas se presenta la anatomía a simple vista de la médula espinal, cabeza y cuello, como preparación para una exposición de seis semanas sobre neurobiología. El programa neurobiológico comprende neuroanatomía, neurofisiología, neuroquímica y neurología clínica en una unidad efectiva que establece referencias particulares con pacientes enfermos con afecciones que

ilustran y correlacionan las áreas de estudio con las diferentes disciplinas involucradas.

S U M A R Y

With the initiation of instruction at the new School of Medicine of the University of New Mexico in 1964, the structural aspects of medical biology was programmed as an interdisciplinary presentation.

During the first eight weeks, basic concepts of Gross Anatomy are introduced by way of the dissection of the extremities. Concurrently, the microscopic anatomy of cells and basic tissues are studied in close association with the presentation of biochemical principles.

Following these concept introductions, a two week period is devoted to the red blood cell. Glycolysis, hemopoiesis, hemoglobin synthesis and selected metabolic influences concerned with red cell function are considered. Lymphatic organs related to immunologic phenomena are briefly presented in conjunction with a three week exposure to microbiology. A short consideration of contraction and conduction properties in muscle and nerve respectively precedes the beginning of integrated organ system biology.

Cardiovascularpulmonary biology forms a six week interdisciplinary teaching bloc, followed by two weeks of renal biology, three weeks of gastrointestinal biology and four weeks of endocrine biology. In all of these subject areas, gross, microscopic, ultrastructural and developmental aspects of anatomy are represented as integrated components with the physiological, biochemical and pharmacological presentations.

Gross Anatomy of spinal cord and head and neck are presented in three weeks in preparation for a six week exposure to Neurobiology. The neurobiological program

offers neuroanatomy, neurophysiology, neurochemistry, neuropharmacology and clinical neurology in an effective unit which features particular reference to patients with disorders that illustrate and correlate areas of study of the several disciplines involved.

The University of New Mexico School of Medicine initiated instruction in Medical Biology to its first class of 24 medical students in September 1964. Our intent in the design of this first year curriculum was to present the several basic medical sciences in an interdisciplinary fashion in close association with an early exposure of students to patients in the hospital setting.

The students take two courses during the first year, Medical Biology and Clinical Science. Into Medical Biology is structured the subject matter and participation of the Department of Anatomy, Biochemistry, Microbiology, Pharmacology and Physiology. In Clinical Sciences, an introduction to normal human behavior is correlated with the beginnings of patient interviews in the hospital during the first 15 weeks. The remaining 23 weeks are concerned with an introduction to physical diagnosis and history-taking.

The first eight weeks of the curriculum can be thought of as an introduction to the principles of molecular, cellular and tissue biology by exposure of students to increasingly complex levels of organization. Thus atomic and elemental principles are considered before the submicroscopic features of biological systems are introduced. This pattern then extends to cellular and tissue biology. Of interest in our sequence is the fact that biochemical principles are present from the point of view of proteins, their composition, activity, formation and metabolism. This permits a close association in teaching those features of biological structure in which spe-

cific proteins can be identified and the interrelationships between the disciplines are thereby reinforced. Such areas of practical reinforcement in our program are found in considerations of the types of collagen of connective tissues, certain active amines of connective tissue cells and neural elements, the proteinaceous coatings of cells and their relationship to tissue fluid, muscle proteins and the structure of membranes.

In addition to chemical and morphological expositions during the introductory eight weeks, genetics and biometrics are programmed in sufficient amounts to provide a substantive foundation on which future presentations are able to build.

During this interval of the first eight weeks, the Gross Anatomical features of the extremities are studied with emphasis on the macroscopic aspects of the basic tissues. Our approach in Gross Anatomy is designed to consider the functionally important relationships and actions of specific areas, rather than detailed discourse in depth. The instructors who are involved in the teaching of morphology during this period have the opportunity to correlate the gross, microscopic and submicroscopic relationships of the basic tissues into a functionally-oriented presentation. This horizontal approach to the presentation of biological structure is an old system used by many earlier Anatomists, but its significance here is that the morphology is considered from a functional basis in biochemical or physiological terms where appropriate.

In the next two weeks the red-blood cell is considered as an integrated unit. Its development and varying structure are studied and following this, the biochemical attributes of glycolysis, oxygen transport and membrane permeability phenomena are dealt with. Selected hemoglobinopathies that show either genetic or adaptive expressions are outlined and where possible, patients having certain blood dyscrasias are presented to illustrate

specific areas in this phase of the program.

After the red-blood cell unit is completed, a two and one half week exposure to the principles of microbiology is begun through the intermediary of nucleic acid metabolism. The anatomy of the nucleus and cytoplasm of mammalian cells is contrasted with the homologous materials of microbial life.

A one week interdisciplinary consideration of membrane phenomena is next programmed using muscle and nerve as the functional biological units. Conduction, excitation, contraction, relaxation and polarization phenomena are surveyed in preparation for intensive study of the biology of the organ systems.

Organ system biology takes on a slightly different aspect. The Gross Anatomy of the area usually precedes the interdisciplinary (microscopic anatomy, physiology, biochemistry and introductory clinical aspects) approach to subject areas. Thus, the next five to six weeks constitute an intensive study of cardiovascular-pulmonary biology. The Gross Anatomy of the thorax is covered, and the structure of the heart, great vessels and respiratory system is interdigitated with the contributions of the other disciplines. An animal heart is provided to each student for a thorough dissection and understanding of its organization.

A two-week period of renal biology is juxtaposed to pulmonary biology because the common properties of acid-base balance are better considered closer together.

Gross Anatomy of the anterior abdominal wall and inguinal region is begun in anticipation of a two and a half to three week consideration of gastrointestinal biology. The structure of the abdominal contents is presented at all levels of organization in conjunction with appropriate physiological and biochemical data.

The next section lasting approximately four weeks is Endocrine Biology and features the Gross Anatomy of the pelvis and perineum, the genital systems of both sexes in microscopic anatomy and the structure and function of all the remaining endocrine glands. Experiments in bioassay of unknown endocrine preparations are used to provide interest in learning the effects and interrelationships of the several glands under consideration.

The curriculum continues with approximately three weeks of the Gross Anatomy of the spinal cord, back, ad head and neck. This intensive study of Gross Anatomy is gradually diminished to provide time to begin readings in programmed neuroanatomy (Sidman & Sidman). In the latter sessions, specific demonstrations of brain structures are performed with small groups of students. Thus the gross anatomical foundation to proper orientation of students to neurobiology is established by close positioning of these importantly related subject areas within the curriculum. Approximately six to seven weeks of neurobiology concludes the first year. This integrated presentation consists of neuroanatomy, neurophysiology, neurochemistry, neuropharmacology and clinical neurology. Each week specific patients are presented to illustrate interesting aspects of the neurobiological spectrum.

It was mentioned earlier that with the beginning of organ system biology, the clinical experiences of the students expand to include the fundamentals of physical diagnosis. Such applied knowledge in the first year has a positive effect on understanding the structural basis for the physical examination and makes the overall efforts in instructing students morphologically, immesasurably easier.

Our teaching plan permits instructors to follow their presentations to students from the gross to the microscopic aspects of the particular area with which they may be concerned. This teaching scheme has two principal effects on the shedule: (1) it

permits continuity of the anatomical discipline within the interdisciplinary approach, and (2) it eases the time commitment of the instructors to identifiable blocks of the program. Thus as mentioned earlier, individuals concerned with teaching cytology and basic tissues will also cover the anatomy of the extremities. Those concerned with the gross anatomy of the thorax and abdomen will handle the corresponding microscopic study of the tissues and organs involved here. Instructors concerned with the pelvis and perineum will be involved in the endocrine biology areas of the program. Similarly, the neurobiologically-oriented teachers will have primary concern for the Gross Anatomy of the head and neck and spinal cord so that effective translation of principles between the two areas of anatomy will form a logical transition.

Appended below is a synopsis of our next year's curriculum showing the distribution of subject areas and their relative time allotments. For those interested in our experiment in integrated teaching, we have a supply of detailed copies of our first year curriculum for 1966-67 which are available upon request.

THE UNIVERSITY OF NEW MEXICO SCHOOL
OF MEDICINE
CURRICULUM FOR FIRST YEAR 1966-1967

**A synopsis of departmental participation
in integrated instruction**

**Approx.
hrs.**

Orientation week September 8-10:

Weeks 1-8, September 12-November 5:

Basic principles underlying molecular, cellular and tissue biology

1. The principles of chemical behavior from the atom to

	Approx. hrs.
complex proteins - including considerations of physical chemistry, pK's photometry, counter-current distribution, chromatography, electrophoresis, enzymes, energetics, isotopes and macromolecules	97
2. Gross anatomical dissection of upper and lower extremities	50
3. Cytology and general histology - covering all the basic tissues and including histochemical and cytochemical correlations	35
4. Biometrics	6
5. Genetics - programmed instruction with lectures	12
6. Pharmacological principles	3
7. Clinical sciences	48
8. Group conferences	21
9. Unprogrammed time	72

Weeks 9-10, November 7-19:

Biology of Red Blood Cell

1. Biochemistry related to glycolysis, hemoglobin synthesis and membrane phenomena	33
2. Morphology of blood and marrow	33
3. Pathology	13
4. Clinical sciences	6
5. Review sessions	12
6. Unprogrammed time	4
	18

Weeks 11-13, November 21-December 10:

Introduction to Microbiology

1. Principles of Microbiology, including considerations of nucleic acids, microbial anatomy and physiology, nutrition, growth and metabolism. Selected experiments in the laboratory	56
2. Student Seminars	10

	Approx. hrs.
3. Group conferences	4
4. Clinical sciences	14
5. Unprogrammed time	24

Week 14, December 12-17:

General membrane phenomena and muscle

1. Muscle and membrane phenomena (Physiology)	18
2. Microscopic anatomy	1
3. Pharmacology	1
4. Gross Anatomy - thorax	8
5. Clinical sciences	6
6. Unprogrammed time	9

Weeks 15-20, December 19-February 4:

4:

(Christmas holiday December 22/January 4)

Cardiovascular-pulmonary biology

1. Physiology	73
2. Gross Anatomy-complete thorax	15
3. Microscopic Anatomy	14
4. Pharmacology	6
5. Review	9
6. Conferences	7
7. Clinical sciences	30
8. Unprogrammed time	62

Weeks 21-23, February 6-21:

Renal biology

1. Physiology	53
2. Microscopic Anatomy	5
3. Review	3
4. Conferences	4
5. Clinical sciences	14
6. Unprogrammed time	24

Weeks 23-25, February 22-March 11:

11:

	Approx. hrs.
Gastrointestinal biology	
1. Gross Anatomy	35
2. Microscopic Anatomy	11
3. Physiology	18
4. Biochemistry	17
5. Conferences	3
6. Clinical sciences	16
7. Unprogrammed time	14

Weeks 26-29, March 13-April 8
(Easter Vacation March 22-28)

Endocrine Biology

1. Gross Anatomy	30
2. Physiology	28
3. Microscopic Anatomy	27
4. Biochemistry	6
5. Student reports of hormone unknowns	3
6. Clinical sciences	18
7. Unprogrammed time	18

Weeks 30-32, April 10-29:

Gross Anatomy of Back, Spinal Cord, and Head and Neck (Introduction to Neurobiology)

1. Gross Anatomy	74
2. Programmed Neuroanatomy	10
3. Clinical sciences	18
4. Unprogrammed time	27

Weeks 33-38, May 1-June 9:

Neurobiology

1. Programmed Neuroanatomy	18
2. Neuroanatomy	44

	Approx. hrs.
3. Neurophysiology	60
4. Neuropharmacology	4
5. Neurochemistry	6
6. Clinical sciences, patient presentations and review	52
7. Unprogrammed time	63

Summary of the Department of Anatomy participation in the first year curriculum by distribution of hours:

Gross Anatomy	212 hrs.
Microscopic Anatomy	106 "
Neuroanatomy, incl. programmed text	72 "
Total:	390

Approximate hours of participation by disciplines in the first year curriculum:

	hrs.
Anatomy	390
Biochemistry	159
Physiology	250
Microbiology	56
Other disciplines	38
Group conferences and review	68
Clinical sciences	228
Sub-total	1,189
Unprogrammed time (22% of total time)	331
Total	1,520

Parallelisms in fiber relations and variations in nuclear patterns in the phylogeny of the amygdala¹

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RESUMEN

H. N. Schnitzlein

La amígdala de la lamprea está representada por una sola masa amigdalina, en el amplio hemisferio indiferenciado. En peces y anfibios hay dos divisiones de la amígdala, una corticomedia y una basolateral.

En reptiles y mamíferos la amígdala ha pasado ya por una diferenciación más amplia encontrándose los siguientes núcleos; lateral, basal, cortical y medial, así también como una región amigdalina anterior, un núcleo central y en algunos reptiles y mamíferos hay un núcleo adicional.

Las aves representan una rama de alta especialización de la línea de los reptiles y no han sido tomadas en cuenta en este trabajo.

El tracto olfatorio lateral que se deriva del bulbo olfatorio y el bulbo olfato-

rio accesorio (frecuentemente hay una sinapsis a través de la substancia gris al lado del tracto o en el área amigdalina anterior) termina ampliamente en el núcleo medial y cortical de la amígdala. El complejo basolateral está característicamente interconectado con la amígdala contralateral a través de la comisura anterior (fig. 13). Los núcleos basal y lateral también tienen interconexiones con la corteza suprayacente. La diferenciación aumenta en la escala filogenética también con la diferenciación de las áreas corticales vecinas (fig. 13).

Ambos núcleos basolateral y corticomedia contribuyen a formar la estría medular y la estría terminal interconectándolas con los centros epitalámicos e hipotalámicos (fig. 13).

En la escala filogenética primero hay una migración parcial de las células periventriculares que representan un complejo amigdalino primario que está lejos del ventrículo. Por lo tanto, las masas

¹ This investigation was supported in part by research grants (NB04295, NB01804, and NB05250) from the National Institute of Neurological Diseases

and Blindness, the National Institutes of Health, U. S. Public Health Service.

SUMMARY

H. N. Schnitzlein

que se forman secundariamente cambian su posición como resultado del aumento de desarrollo de otras áreas corticales y estriadas. Este cambio o rotación se lleva a cabo en dos direcciones: lateral a medial y caudal a cefálica. En la fig. 14 se observa claramente la rotación de los núcleos laterales, de la amígdala a una posición como se lleva a cabo en los reptiles. En los mamíferos se observa la rotación a una posición ventro lateral (aunque manteniendo su relación ventricular). La rotación de las porciones corticales y mediales de la amígdala se observa en reptiles y mamíferos inferiores. En el hombre se observa el cambio de la posición ventral o una más dorsal.

Filogenéticamente hay una tendencia del núcleo basolateral de alargarse caudalmente un poco hacia la masa principal del núcleo córtico medial, además en formas superiores el complejo basolateral aumenta marcadamente en tamaño en relación al córtico medial. La amígdala completa cambia de posición facialmente y rota con el crecimiento del polo temporal. La relación del grupo basolateral con la comisura anterior aparece para permanecer constante.

La armazón de las conexiones fibrosas a través de la escala filogenética se establecen también y son constantes.

Los tipos celulares y las relaciones entre las porciones córtico medial y basolateral, son también características.

La diferenciación anatómica que se observa en los mamíferos lo mismo que la rotación están indudablemente asociados a los cambios de otras áreas del cerebro especialmente con las de la corteza. Aunque estas homologías son evidentes, es también obvio que las funciones de los núcleos del complejo amigdalino pueden variar en relación con el aumento o disminución en tamaño, especialidad y complejidad de las áreas corticales estriadas y otras regiones con las cuales estos núcleos están interconectados.

The amygdala of the lamprey is represented by a single amygdaloid mass in the largely undifferentiated hemisphere. In fish and amphibians, two divisions of the amygdala, a corticomедial and a basolateral, have been recognized. The amygdala has undergone further differentiation in reptiles and mammals and separate lateral, basal, cortical and medial nuclei (as well as an anterior amygdaloid region, a central nucleus and, in some reptiles and mammals, additional nuclei) have been identified. The birds represent a highly specialized side branch of the reptilian line and have not been considered in this abbreviated presentation.

The lateral olfactory tract, arising from the olfactory bulb and the accessory olfactory bulb (frequently with a synapse in course in the gray along the tract or in the anterior amygdaloid area), terminates largely in the cortical and the medial nuclei of the amygdala (Fig. 13). The basolateral complex is characteristically interconnected with the contralateral amygdala through the anterior commissure (Fig. 13). It is also the basal and the lateral nuclei that have the most prominent interconnections with the overlying cortex and further differentiate and increase in phylogeny with the increase in the differentiation of these related cortical (and striatal) areas (Fig. 13). Both the basolateral and the corticomедial nuclei contribute to the stria medullaris and to the stria terminalis, interconnecting them with epithalamic and with hypothalamic centers (Fig. 13).

In phylogeny, first there has been a partial migration of the periventricular cells representing a primordial amygdaloid complex away from the ventricle. The masses thus formed secondarily shift their position as the result of the increasing development of other cortical and or

striatal regions. This shift or rotation takes place in two directions —lateral to medial, and caudal to rostral. Evident in figure 14 is the rotation of the lateral nucleus of the amygdala from a dorsal position, as seen in the reptile, to a ventrolateral location (although maintaining its ventricular relationship) in the mammal and of the cortical and medial portions of the amygdala of reptiles and lower mammals from a ventral position to a more dorsal and medial location in man. Phylogenetically, there is a tendency for the basolateral nuclei to extend slightly caudal to the main mass of the cortico-medial nuclei. Moreover, in higher forms, the basolateral complex is markedly increased in size with reference to the corticomedial group. The entire amygdala is shifted rostrally and rotates with the growth of the temporal pole. The relationship of the basolateral complex to the anterior commissure appears to remain constant.

The framework of the fiber connections is consistent throughout phylogeny. The cell types and the relationships are also characteristic for the corticomedial and the basolateral divisions. Anatomical differentiation within the mammalian phylum and the rotation and the differentiation observed are undoubtedly associated with the changes in other brain centers, most particularly with those of the neocortex.

Although these anatomical homologies are evident, it is equally obvious that the functions of the nuclei of the amygdaloid complex will vary with the relative increase or decrease in size and especially in complexity of those cortical, striatal, and other regions with which these nuclei are interconnected.

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INTRODUCTION

It is the intent of this presentation to summarize some of the anatomical homologies which have been recognized in the amygdala of various vertebrates. It is obviously impossible to include in this brief presentation representatives of all of the vertebrate orders or to cite the many investigators who have contributed to our knowledge of the amygdaloid complex.

Although general agreement on the anatomical homologies of the various amygdaloid nuclei is lacking and the details of the specific connections of the amygdaloid nuclei of the more differentiated mammalian amygdalae are incompletely known, certain anatomical considerations indicate parallel evolutionary development in the various vertebrate orders.

In order to establish nuclear and fiber homologies, three criteria have been used: 1) the location and the general relations; 2) the histology and the cytology; 3) the connections, both afferent and efferent.

AMYGDALA OF FISHES

H. N. Schnitzlein

Those vertebrates which are usually considered to be fish have developed along several separate evolutionary lines. These fish include the **Cyclostomata**, the **Chondrichthes**, the **Actinopterygii**, the **Dipnoi**, and the **Coelacanthini**. Obviously then, great variation will occur in these divergent fish. Only two will be represented in this presentation, the catfish, a teleost, one of the actinopterygian fishes, and **Protopterus**, a representative of the dipnoan lungfish. The teleosts, like other actinopterygian fish, have solid hemispheres. An area along the ventrola-

teral portion of the hemisphere has been recognized as a primordial amygdala (Weston, 1937; Droogleever Fortuyn, 1961). This homology has been denied by some (Cage, 1893; Holmgren, 1922; Nieuwenhuys, 1962). Different nuclear areas in the lateral portion of the telencephalon have been pointed out by others (Miller, 1940) who have not attempted homologies.

In the catfish, the most rostral part of the ventrolateral region of the telencephalon is represented by an anterior amygdaloid area with the typical relations to the lateral olfactory tract and to the piriform cortex. More caudally, two nuclear groups can be observed in the catfish, a primordial corticomедial group (fig. 1A) with smaller cells and with relations to the lateral olfactory tract, and a larger-celled portion, more intimately related to the striatum, which has been called a primordial basolateral amygdaloid complex (Fig. 1A). The corticomедial group diminishes in size caudally; the basolateral portion increases in size at caudal levels. The relationships of the primordial basolateral amygdala to the primordial general pallial area and to the primordial hippocampal formation, which is along the dorsomedial aspect of the telencephalon, become evident at the caudal pole of the hemisphere.

The corticomедial part of the amygdala receives the lateral olfactory tract and its neurons project into the stria medullaris by way of a lateral corticohabenular tract (Fig. 2A) and, through the stria terminalis (Fig. 2A), into the anterior hypothalamic and preoptic areas of the same side and (through the anterior commissure) to these areas on the opposite side. The basolateral portion of the amygdala has true inter-amygdaloid commissural fibers crossing through the anterior commissure. The basolateral amygdala also contributes to the stria terminalis and projects to the habenula by way of the lateral corticohabenular component of the stria medullaris. The inter-amygdaloid fibers of the

anterior commissure are differentially stained in pyridine silver preparations and pass ventral to the hippocampal commissure (Fig. 2A).

In the lungfish, **Protopterus**, also, a small anterior amygdaloid area can be recognized at the base of the primordial piriform region. It has the typical relations to the lateral olfactory tract. This fish has lateral telencephalic ventricles; however, the olfactory bulbs are dorsally situated (probably due to the enlarged tuberculum olfactorium) and the relationships of the amygdala are somewhat distorted from the usual pattern. In **Protopterus**, the corticomедial portion of the amygdala (Fig. 1B) lies adjacent to the ventricle, more ventral in position than the larger-celled basolateral part (Fig. 1B), which is also periventricular but more dorsally situated. The corticomедial group is related to the primordial piriform region and to the anterior amygdaloid area. The primordial basolateral amygdala is related to the primordial general pallial region and, more caudally, to the primordial hippocampal formation. The periventricular position of the basolateral group is more evident in caudal sections where this group is larger and its relationship to the primordial hippocampal formation becomes more evident.

The lateral olfactory tract arises from olfactory bulb and from the accessory olfactory bulb in the lungfish. A distinct accessory olfactory bulb has not been identified in the catfish. The lateral olfactory tract of the catfish terminates in the primordial corticomедial nuclear group and in the piriform region. In the lungfish, the lateral olfactory tract courses ventrally around the hemisphere due to the dorsal positioning of the olfactory bulbs. This tract extends to the lateral and intermediate olfactory tubercular areas; some fibers however, sweep off into the amygdala. These pass through anterior amygdaloid area into the corticomедial group. Other fibers, probably those arising from the accessory olfactory bulb,

may terminate in the basolateral amygdala or may course through it to the corticomедial nuclear group.

A separate component of the anterior commissure (Fig. 2B) interconnects the basolateral nuclei of the two hemispheres in **Protopterus**, as in the catfish (Fig. 2A) and can be recognized by its differential staining in pyridine silver preparations. The stria terminalis arises from both the corticomедial and the basolateral parts of the amygdala in the lungfish and the catfish and terminates in the ipsilateral and contralateral preoptic and anterior hypothalamic areas. The decussating component of stria terminalis courses ventrally in the anterior commissure of the catfish (fig. 2A) and crosses more caudally than the levels shown in figure 2B in the African lungfish. The lateral corticohabenular component of the stria medullaris (Fig. 2B) may also be identified at levels through the caudal part of the anterior commissure in the lungfish as it arises from both the primordial corticomедial and the primordial basolateral nuclear groups. This component of the stria medullaris sweeps out of the amygdala into the diencephalon and is situated along the lateral aspect of the stria medullaris as it enters the habenula.

The amygdala of the fish, then, has two major subdivisions: a primordial corticomедial and a primordial basolateral portion recognizable by their position and their relationships to other telencephalic nuclei and particularly by their connections. In some fish, such as the catfish, these areas have migrated into the lateral telencephalic wall; in other fish, such as the African lungfish, **Protopterus**, they are represented in the periventricular gray.

AMYGDALA OF AMPHIBIANS

H. H. Hoffman

Herrick (1921) described the amygdala of *Rana pipiens* as "one of the most

clearly defined regions of the anuran brain" but pointed out no subdivisions in the area. Röthing (1926) emphasized the relation of the amygdala to the ventricle and illustrated the ventricular ridge formed by it in **Rana fusca**. In the anuran material available, as in tailed amphibians (Herrick, 1933), the amygdala is gray which forms an eminence on the ventricular wall. However, in anurans, this gray can be further subdivided into the two portions characteristic of many vertebrate forms a corticomедial and a basolateral subdivision (Hoffman, 1963, 1966a, 1966b; Howell and Hoffman, . . . 1966). The corticomедial subdivision in anurans, which have a marked development of the medial rather than the lateral hemisphere wall, is on the whole, the more extensive part of the amygdala and forms all of the ventricular eminence rostrally. Nevertheless, as anterior commissure levels are approached (Figs. 3A, B, and C), the basolateral subdivision, which lies ventral and medial to the corticomедial subdivision, becomes prominent and occupies a position between the medial part of the corticomедial nuclear group and the ventricular wall. In the brain of **Xenopus leavis**, but less noticeably in those of other tailless amphibians available for study, in addition to these two main subdivisions, and perhaps to be allocated to the corticomедial part, is a differentiable mass of somewhat scattered cells. This mass lies in relation to the fibers of the lateral olfactory tract which is regarded as an anterior amygdaloid area.

To be emphasized is the close relationship of the corticomедial amygdaloid group to the hippocampal formation at commissural and postcommissural levels (Figs. 3A, B, and C). The merging of the piriform cortex with the basolateral part of the amygdala, which is seen in many vertebrates, is questionable in the available anuran material but striatal-amygdaloid continuity is established by scattered nerve cells extending between the two areas.

Certain basic fiber connections of the amygdaloid complex have been described by Herrick (1921) in *Rana pipiens*, by Röthing (1926) in *Rana fusca*, by Herrick (1933) in *Necturus*, and by Hoffman (1963) in the *Ranidae* and in *Bufo marinus*. These include components of the lateral olfactory tract, of the stria medullaris, of the stria terminalis (together with the olfactory projection tracts), and of the anterior commissure. Mentioned also are interconnections of the amygdala with the hippocampus and with the striatum.

The components of the lateral olfactory tract to the amygdala arise from both the olfactory formation and the accessory olfactory bulb. In *Pipa pipa* there are three clear cut portions of this lateral olfactory tract of which the middle part arises from the accessory olfactory bulb. This material, then, provides information concerning the distribution of the lateral olfactory tract. The fibers from the olfactory formation distribute primarily, but not exclusively, to the corticomedial subdivision of the amygdala and those from the accessory olfactory bulb, primarily at least, to the basolateral part.

The stria terminalis can be traced from the corticomedial, and to a somewhat less extent, from the basolateral subdivision of the amygdala, (Figs. 4A and B.) to the preoptic and anterior hypothalamic areas of both sides. The contralaterally distributing fibers cross in the anterior commissure. The components of the stria terminalis (and probably of the associated olfactory projection tracts) provide interconnections between the amygdaloid area and septal, preoptic and hypothalamic regions.

The interamygdaloid component of the anterior commissure is truly commissural, interconnecting the basolateral subdivisions of the two hemispheres (Figs. 4A and B). This is a prominent feature of the fiber connections in anurans and is especially marked in *Pipa pipa* (Fig. 4A).

The so-called amygdalohabenular component of the stria medullaris is part of

the lateral corticohabenular tract (Fig. 4A). It seems quite probable that some of these fibers may be the commissural, interconnecting piriform lobe and amygdaloid areas of the opposite sides of the brain.

AMYGDALA OF REPTILES

H. N. Schnitzlein

In reptiles, there are corticomedial and basolateral subdivisions of the amygdala, as well as an anterior amygdaloid area which may well be considered with the corticomedial group. These subdivisions are differentiable into secondary nuclear masses. Where, from general character, position and connections, such masses appear to be comparable to the amygdaloid nuclei of mammals, the mammalian name has been used (as Johnston, 1923, did) for the reptilian nuclear groups. Where homologies have not been attempted, letters (such as L and M, Fig. 5) have been employed.

In the snake, as in various other reptiles, the deep structures in the lateral hemisphere wall bulge out into the lateral ventricle, forming a hypopallial eminence (Elliot Smith, 1919) or a dorsal ventricle ridge (Johnston, 1913, 1923). This ridge divided by an oblique sulcus into a rostral (or rostral part of the) and a caudal (or caudal part of the) dorsal ventricular ridge. The rostral part is formed by a striatal mass; the caudal part is an amygdaloid ridge.

The most conspicuous element beneath the amygdaloid ridge of the snake is an ovoid mass (Figs. 5-7), the walls of which are formed by deeply staining, closely arranged cells, with a central part, an essentially acellular core, composed of fibers which pass to and from the boundary cells. Since this ovoid structure is set somewhat obliquely to the transverse axis of the area, rostralward the boundary cells form an inverted "U" (Fig. 5B) but farther caudalward a more nearly com-

plete ovoid structure (Fig. 7), which terminates most caudally in a cell mass.

The rostral and dorsal part of the ovoid mass of cells in the amygdaloid ridge constitutes a lateral amygdaloid nucleus (Figs. 5-7). This nucleus has the two connections which are considered to give it special significance in various forms; that is, (a) it is connected with the corresponding amygdaloid area of the other side of the brain by the inter amygdaloid component of the anterior commissure (Fig. 6) and (b) it is also interconnected with the piriform cortex by association fibers and by merging gray (fig. 6). These connections characterize the lateral nucleus in mammals. In addition, the reptilian lateral nucleus, like its mammalian homologue, contributes to the stria terminalis (fig. 6), making interconnections through this system with septal, preoptic and hypothalamic regions.

The basal nuclear complex (fig. 5B), identified in the turtle by Johnston (1923) and in the snake by Carey (1966) and by Crosby et al. (1966), may well contain, in the more scattered cells along its ventral border, elements of the accessory basal nucleus. It receives fibers from the lateral olfactory tract (probably from the accessory olfactory bulb). The basal nuclear complex contributes fibers to the stria terminalis and to the stria medullaris (fig. 6) and is related to the piriform cortex by association fibers and cell strands (Fig. 5B).

In addition to the lateral nucleus and the basal nuclear group, there are several cell clusters represented in figure 5B by the letters "L" and "M" which, from their positions and connections, appear also to belong to the basolateral complex. However, their homologies, if such exist, are not known and they may be strictly reptilian nuclear differentiations.

The anterior amygdaloid area (Fig. 5A) lies at the rostral end of the amygdala, being merged with cells that accompany the lateral olfactory tract in its course

caudalward through the hemisphere. It probably receives and gives fibers to this tract. It also merges with the nucleus of the diagonal band of Broca and gives to and/or receives fibers from this band. Although it appears ventrally in our snake material, it swings medialward shortly into the position typical for it in lower mammals. A well-differentiated nucleus of the lateral olfactory tract is not demonstrable in the available material. Possibly, this is associated with the larger size of the accessory olfactory bulb and the reduced amount of olfactory in these forms (Carey, 1966).

All the reptilian material consulted showed a medial amygdaloid nucleus along the ventromedial hemisphere wall. This nucleus receives a very few lateral olfactory fibers, but is characterized particularly by its contributions to the stria medullaris [lateral corticohabenular, caudal part. (Fig. 7)] and to stria terminalis (Fig. 6). The lateral corticohabenular tract has commissural fibers that cross in the habenular commissure but interconnect hemisphere areas and, probably, has also some fibers to the habenulae of both sides. The stria terminalis relates the medial amygdaloid area with the septal areas bilaterally and with preoptic and hypothalamic centers on both sides of the brain.

The cortical amygdaloid nucleus can be identified at more caudal amygdaloid areas as the ventral part of the ovoid cell mass which is close to the ventral surface of the brain. Followed forward, the boundary cells of the ovoid mass disappear in part, leaving the very evident inverted "U" of the lateral nucleus and a ventral band which continues forward as a distinct and characteristic cortical nucleus. The cortical nucleus receives a few lateral olfactory tract fibers and contributes to the stria terminalis and to the caudal part of the lateral corticohabenular component of the stria medullaris. A central amygdaloid nucleus, as identified by Johnston (1923), is easily identifiable. Its

specific connections are not sufficiently clear in the available material to warrant homologies.

It is evident, then, that not only are the major subdivisions—the corticomедial and the basolateral amygdaloid groups—present in reptiles but that also secondary nuclear differentiation has occurred within this framework that parallels to a very considerable degree that seen in mammals. This parallelism in pattern suggests that such nuclear differentiation occurred in a common ancestral type (which has long since disappeared) and has been handed down in the course of phylogeny to both living reptiles and mammals.

Lack of space prevents consideration of the avian amygdala. Avian forms are still farther away than reptiles from the main stream of evolutionary development.

THE AMYGDALOID COMPLEX OF THE OPOSSUM

E. G. Hamel, Jr.

In the opossum, *Didelphis virginiana*, as in many submammals, the amygdaloid complex can be divided into basolateral and corticomедial portions. The anterior amygdaloid area may be allocated to the latter part. The present terminology follows that established for the opossum by Johnston (1923), Van der Sprenkel (1926), Loo (1931) and Volker and Hamel (1966).

In the rostral portion of the amygdaloid complex, at the anterior commissure level, the nucleus of the lateral olfactory tract has reached its greatest extent and can be separated into medial and lateral divisions (Fig. 8A). The anterior amygdaloid area, which is the undifferentiated gray matter dorsal to this nucleus, merges caudally into the definitive amygdaloid nuclei. In the midportion of the amygdaloid complex (Fig. 8B) most of the other nuclei are represented in their typical relationships. The lateral nucleus is an area of

larger cells bounded laterally by the external capsule and medially by the basal nucleus with smaller, more compactly arranged cells. A small part of the basal accessory nucleus is also present at this level as are the cortical, medial and central nuclei, which represent here the corticomедial portion of the complex. In the caudal part of the amygdala, the lateral ventricle forms a dorsal boundary (Fig. 8C). The lateral nucleus, somewhat reduced in size, produces a small but obvious ridge (suggestive of the reptilian amygdaloid ridge) in the floor of the ventricle. The basal nucleus is smaller than it is more rostrally and the basal accessory nucleus extends partially across its caudal aspect toward the medial wall. The central nucleus decreases caudally and the medial and the cortical nuclei retain their characteristic positions. At the most caudal extent of the amygdala (Fig. 8D), a relationship of this area with the hippocampal formation—but not its complete continuity—is established as the ventral tip of the cornu ammonis turns laterally toward the basolateral component of the amygdala. Caudal to this plane, the gyrus dentatus merges with the medial amygdaloid nucleus.

Projections from the amygdaloid nuclei include fascicles to the stria terminalis, to the stria medullaris and to the interamygdaloid component of the anterior commissure. The stria medullaris (Fig. 9D) forms a large bundle of myelinated fibers composed chiefly of fibers from the cortical and the medial amygdaloid nuclei to the habenula. The stria terminalis (Fig. 9E), originating from the basal, the lateral and the basal accessory nuclei, as well as from the cortical and the medial nuclei, projects rostrally following the ventricular surface of the caudate nucleus. These fibers of the stria interconnect the septum, the preoptic region and the hypothalamus of the same and opposite sides of the brain with the amygdala. The interamygdalar component of the anterior commissure (Fig. 9A) consists of fibers

which project from the lateral nucleus of one side to the corresponding nucleus of the opposite side. This component probably carries also commissural connections between the periamygdaloid piriform cortices of the two sides.

The terminations of the lateral olfactory tract (Fig. 9A) in the amygdaloid complex can be demonstrated in *Nauta* material. Lesions of the rostral part of the olfactory bulb induced degeneration of fibers in this lateral tract which can be traced to the nucleus of this tract (Fig. 10A), and to the cortical (Fig. 10B) and the medial (Fig. 10C) amygdaloid nuclei as well as to the piriform cortex. By contrast, the destruction of the accessory olfactory bulb produces degeneration of fibers of the lateral olfactory tract (Fig. 10D) to the lateral amygdaloid (Fig. 10F) nucleus as well as to the piriform cortex (Fig. 10E). A few degenerated fibers can be followed to the cortical and the medial nuclei.

Corticoamygdaloid projections are of two types: (1) those from neopallial cortex to lateral, basal and possibly basal accessory amygdaloid nuclei, and (2) those from the piriform cortex (Fig. 9B), at least, to the lateral amygdaloid nucleus (Fig. 9C) and perhaps to other nuclear groups. From evidence in the literature, it seems quite certain that amygdalocortical components are also present and that these fascicles have, in general, the character of association fibers.

THE CYTOARCHITECTURE OF THE AMYGDALA

N. G. Ferrar

The amygdaloid nuclear complex has been described in a variety of animals (Herrick, 1921; Johnston, 1923; Crosby and Humphrey, 1941; Brodal, 1947; and Breathnach and Goldby, 1954). The following account is based on the study of characteristic neurons of the amygdaloid complex from representative teleosts (**Ca-**

rassius auratus), amphibians (**Rana pipiens**), reptiles (**Graptemys**), and mammals (**Hapale**). The material was impregnated with 0.75% silver nitrate using the Rapid Golgi technique for fresh material and the Golgi-Cox procedure for formalin-fixed brains. The sections were cut at 80 microns. The locations of these cells and certain cytological characteristics were supplemented by material stained for Nissl granules.

In the goldfish (**Carassius auratus**), the impregnated cells of the basolateral amygdaloid group are of medium size with a wide dendritic spread (Fig. 11A). The cell bodies of such neurons are flask shaped and the dendritic spines are numerous. Nissl preparations through this subdivision of the amygdala show nerve cells varying from large to medium size. The Nissl granules in these nerve cells are medium to small in size and concentrated toward the surface of the cells.

The characteristic nerve cells (Fig. 11C) in the basolateral amygdaloid subdivision of the amygdala of the frog (**Rana pipiens**) are of medium size, but of a characteristically double pyramidal shape. Their dendritic spread is diffuse. Preterminal fibers of tracts entering the nucleus can be seen approaching the dendrites (**arrow** in Fig. 11C). Nissl preparations of these cells reveal a variation in cell size, with larger cells predominating. The Nissl granules are dispersed through the cytoplasm of the cells with a tendency to concentrate near the periphery.

In the turtle (**Graptemys**), the impregnated nerve cells in the lateral amygdaloid nucleus (Fig. 12A) are large and of a double pyramidal shape comparable to those of the basolateral amygdaloid region in the frog. Their dendritic spread is wider, however, and many axons of cells, with cell bodies located in other brain regions, come into synaptic relation with the dendrites and cell bodies of these characteristic neurons of the basolateral complex (**arrow** in Fig. 12A). The Nissl granules are more numerous with an increased ten-

dency to collect at the surface of the cell bodies.

In Golgi preparations, the double pyramidal type of nerve cells, with typically widespread dendrites on which are numerous spines, are easily demonstrable (Fig. 12C) in the lateral nucleus of the marmoset (**Hapale**). The synaptic relations between the incoming axons of distally located nerve cells and the dendritic processes and cell bodies of the double pyramidal cells just described are clearly illustrated in figure 12C where both preterminal (arrow) and terminal (X) fibers can be seen. In Nissl preparations of the lateral amygdaloid nucleus of the marmoset, there are closely arranged, relatively small Nissl granules concentrated near the periphery of the cell bodies.

In contrast to the larger cells (Fig. 11A) characteristic of the basolateral subdivision of the amygdala of the goldfish are the smaller, more nearly round nerve cells (Fig. 11B) of the corticomедial subdivision of the amygdala in this fish. The smaller cells have a limited dendritic spread and very few incoming fibers can be demonstrated. The material stained for Nissl granules shows that these smaller cells have small, densely arranged Nissl granules concentrated toward the periphery of the cell bodies.

The Golgi material of the corticomедial nuclear group in the frog (Fig. 11D) demonstrates nerve cells with small spherical cell bodies having limited dendritic spread. There are relatively few dendritic spines. The Nissl preparations bring out deeply staining, small nerve cells with small Nissl granules collected toward the cell surface.

The medial amygdaloid of the turtle has nerve cells with small, spherical cell bodies (Fig. 12B) The dendritic spread is very limited and the dendritic spines are few. In the Nissl material, the comparable nerve cells show a peripheral distribution of their small, densely arranged Nissl granules.

The impregnated cells of the medial amygdaloid nucleus of the marmoset are small and spherical, with scanty dendrites having few dendritic spines (Fig. 12 D). In fact they are very like those nerve cells demonstrated in the turtle. The Nissl preparation shows the nerve cells of the medial nucleus to be relatively small, deeply stained and with peripherally arranged Nissl granules.

The differences in cellular character between the basolateral and the corticomедial nuclear groups in the various forms studied suggest a specificity as well as a continuity of structural pattern during phylogeny. It seems probable that the characteristic cell type of the basolateral amygdaloid nuclear group reflects both the striatal origin of this group and its functional significance as a vicarious cortex.

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ABBREVIATIONS

amyg.—amygdala
 amyg. ant.—anterior amygdaloid nucleus
 amyg. basal.—basal amygdaloid complex
 amyg. basolat. — primordial basolateral amygdaloid group
 amyg. cort.—cortical amygdaloid nucleus
 amyg. corticomed.—primordial corticomedial amygdaloid group
 amyg. lat.—lateral amygdaloid nucleus
 amyg. med.—medial amygdaloid nucleus
 area preop.—preoptic area
 B - basal amygdaloid nucleus
 B A - basal accessory amygdaloid nucleus
 C - cortical amygdaloid nucleus
 C A - Ammon's horn
 cap. ext.—external capsule
 cap. int.—internal capsule
 Ce - central amygdaloid nucleus
 com. ant.—anterior commissure
 com. ant. amyg. cmpt.—interamygdaloid component of the anterior commissure
 com. ant. sept. tub. cmpt.—septal and tubercular components of the anterior commissure
 com. ant. stria cmpt.—stria terminalis component of the anterior commissure
 com. ant. st. cmpt.—stria terminalis component of the anterior commissure

com. hip.—hippocampal commissure
 com. postop.—postoptic commissure
 fib. ass.—association fibers
 fimb.—fimbria
 F. R.—rhinal fissure
 G. D.—dentate gyrus
 gen. pal.—primordial general pallium
 hab.—habenula
 hip.—hippocampus
 L - lateral amygdaloid nucleus
 l. f. b.—lateral forebrain bundle
 lat. cort. hab. cmpt. st. med.—lateral corticohabenular tract
 M: - medial amygdaloid nucleus
 N. basolat.—basolateral nuclear group of the amygdala
 N. caud.—caudate nucleus
 N. cort.—cortical amygdaloid nucleus
 N. corticomed — corticomedial nuclear group of the amygdala
 N. med.—medial amygdaloid nucleus
 N. lat.—lateral amygdaloid nucleus
 N. tr. olf. lat.—nucleus of the lateral olfactory tract
 nuc. sept. med.—medial septal nucleus
 nuc. preop.—preoptic nucleus
 pir.—primordial piriform lobe
 prim. hip.—primordial hippocampal formation
 prim. pal. dors.—primordial dorsal pallium
 prim. pir.—primordial piriform lobe
 pyr.—piriform cortex
 stria med.—stria medullaris
 stria term.—stria terminalis
 striat.—striatum
 st. term.—stria terminalis
 tub. olf. lat.—lateral part of the olfactory tubercle
 tr. cort. hab. lat. p. caud.—caudal part of the lateral corticohabenular tract
 tr. cort. hab. lat. p. rost.—rostral part of the lateral corticohabenular tract
 tr. olf. lat.—lateral olfactory tract
 tr. op.—optic tract
 tr. preop. hab. med.—medial preopticohabenular tract
 tr. preop. hab. lat.—lateral preopticohabenular tract
 X - terminal synapse

Fig. 1A.

Transverse section through the telencephalon of a
catfish (Thionin stain).

Fig. 1B.

Transverse section through the telencephalon of
Protopterus (Thionin stain).

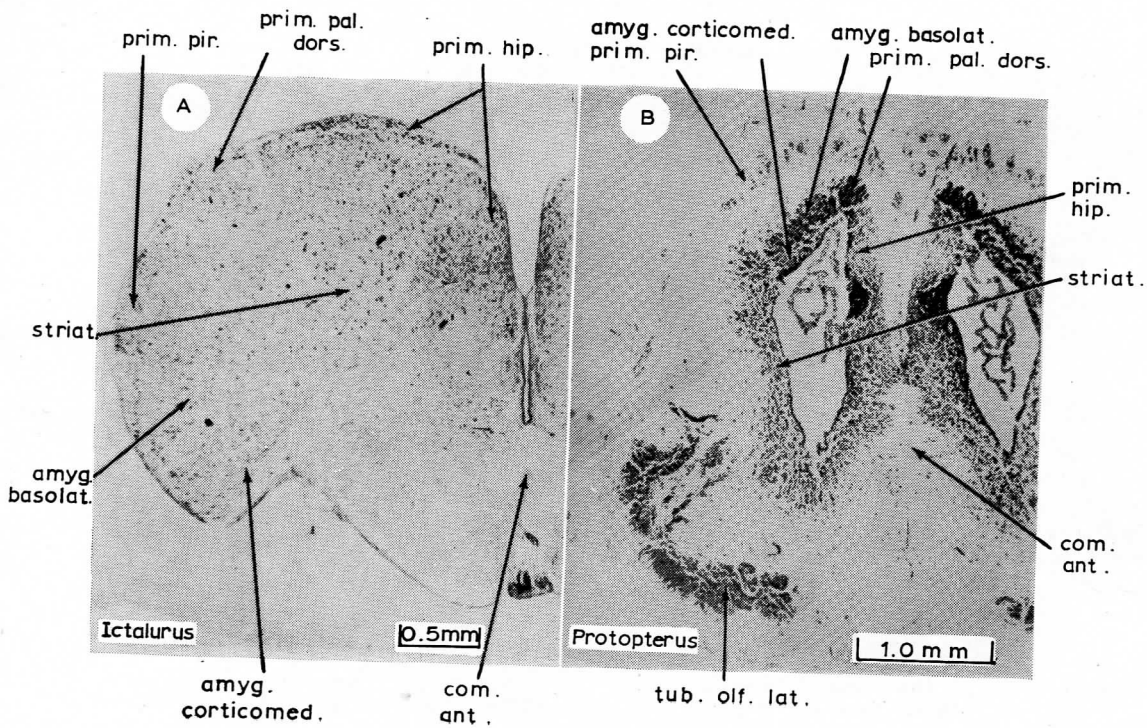


Fig. 2A.

Transverse section through the telencephalon of a catfish (Pyridine silver preparation).

Fig. 2B.

Transverse section through the telencephalon of **Pro-**
topterus (Pyridine silver preparation).

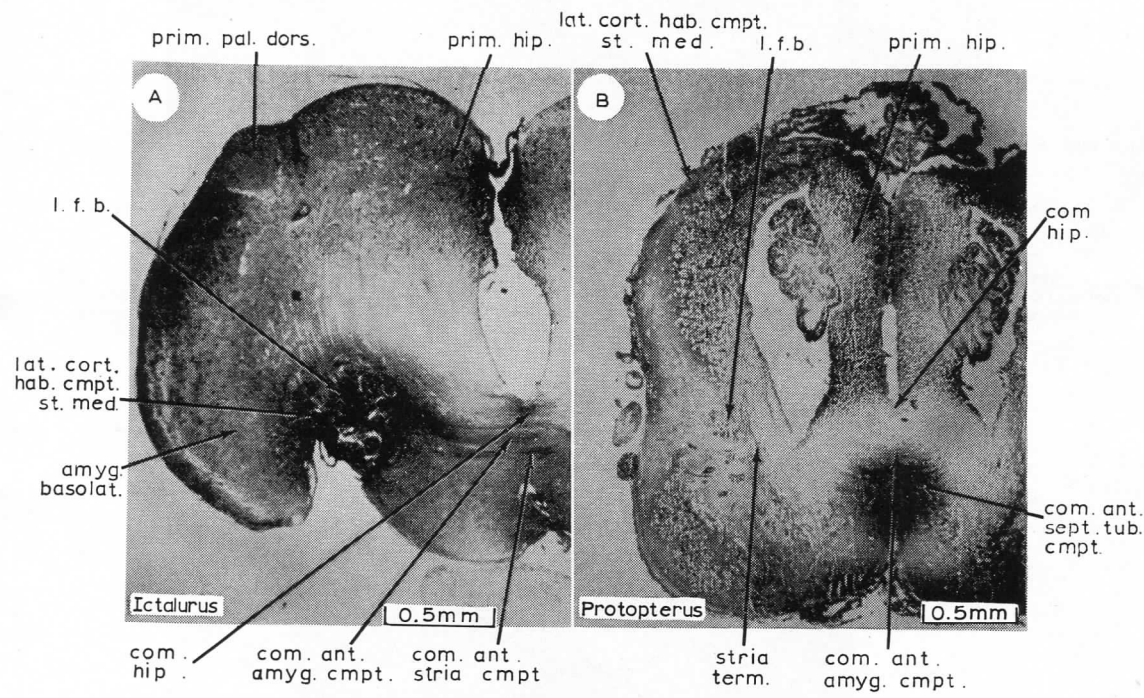


Fig. 3A.

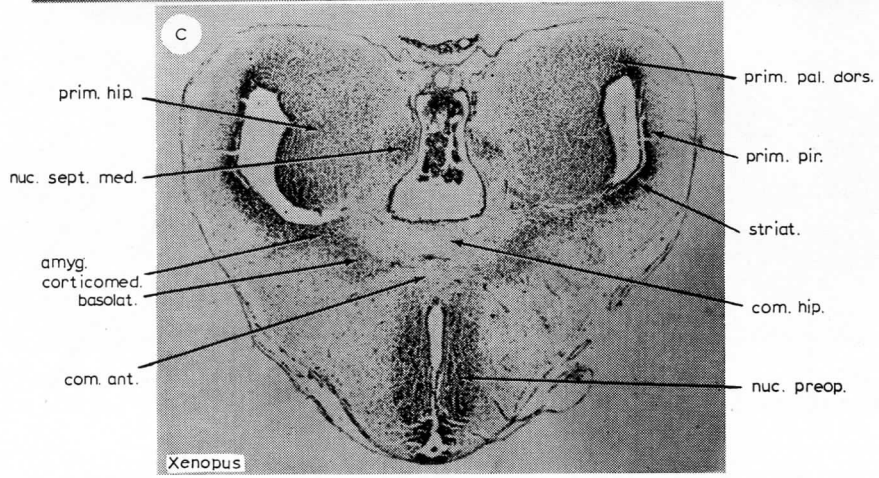
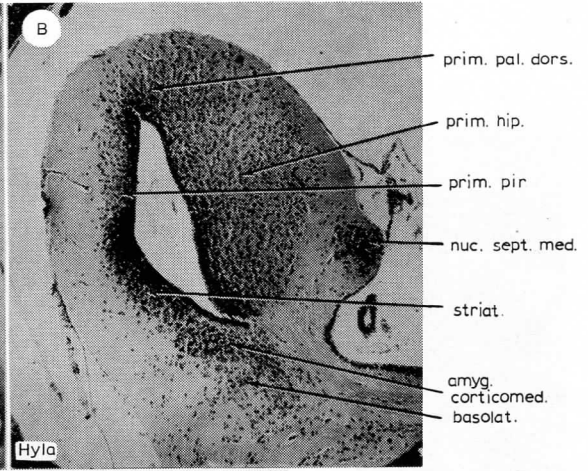
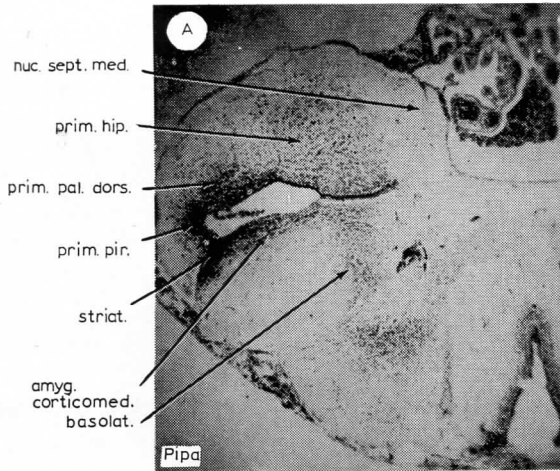
Transverse section through the anterior commissure level of the brain of the aquatic (Surinam) toad, **Pipa pipa** (Thionin stain).

Fig. 3B.

Transverse section through the anterior commissure level of the brain of the tree frog, **Hyla cinerea** (Thionin stain).

Fig. 3C.

Transverse section through the telencephalon (anterior commissure level) of the aquatic frog, **Xenopus leavis** (Thionin stain).



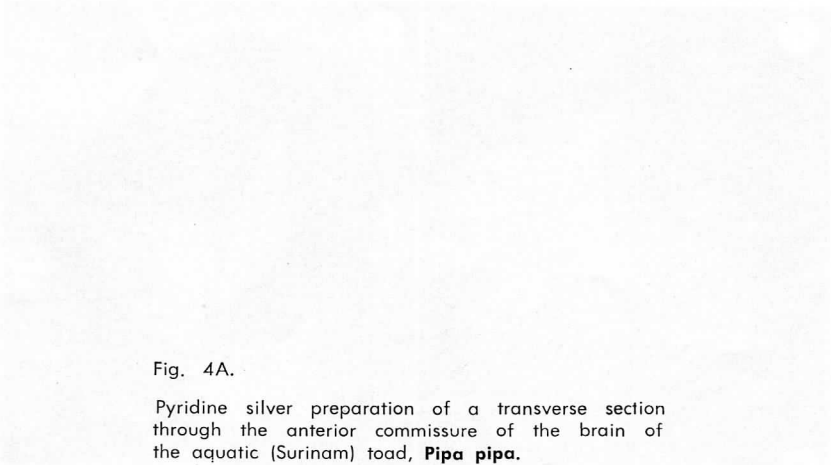


Fig. 4A.

Pyridine silver preparation of a transverse section through the anterior commissure of the brain of the aquatic (Surinam) toad, **Pipa pipa**.

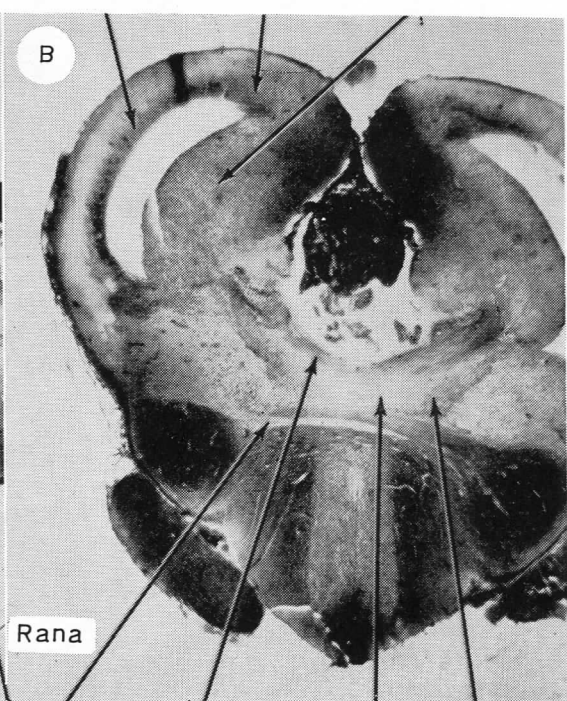
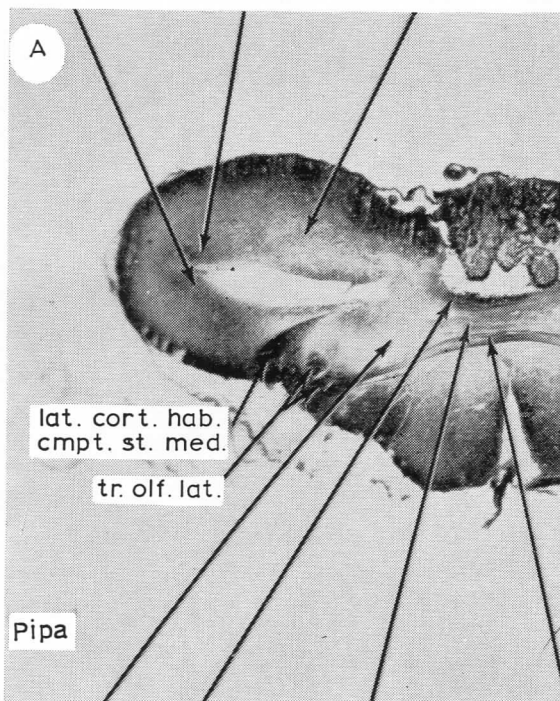
Fig. 4B.

Pyridine silver preparation of a transverse section through the anterior commissure of the brain of the bullfrog, **Rana catesbiana**.



prim. pir. prim. pal.
 dors. prim. hip.

prim. pir. prim. pal.
 dors. prim. hip.



st. term. com. ant. st. cmpt. com. hip. st. term.
com. hip. com. ant. amyg. cmpt. com. ant. st. cmpt.

Fig. 5A.

Photomicrograph of a transverse section of the telencephalon of the rat snake at the level of the rostral part of the anterior commissure (Thionin stain).

Fig. 5B.

Transverse section of the telencephalon of the rat snake caudal to the anterior commissure (Thionin stain).

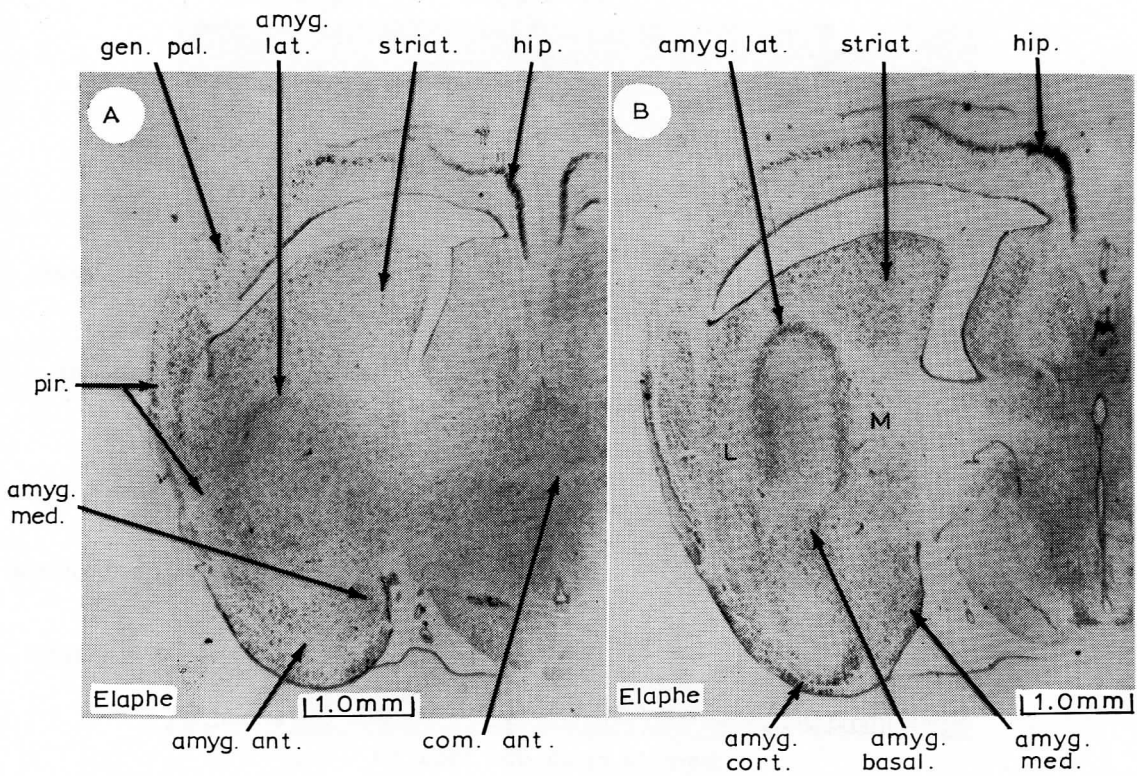


Fig. 6

Drawing of a transverse section through the telencephalon at the level of the caudal part of the anterior commissure of the rat snake. (Pyridine silver preparation).

com. ant.
stria cmpt.

com. ant.
amyg. cmpt.

amyg. lat.

fib. ass.

stria term.

amyg. med.

amyg. cort.

com. hip.

stria term.

l.f.b.

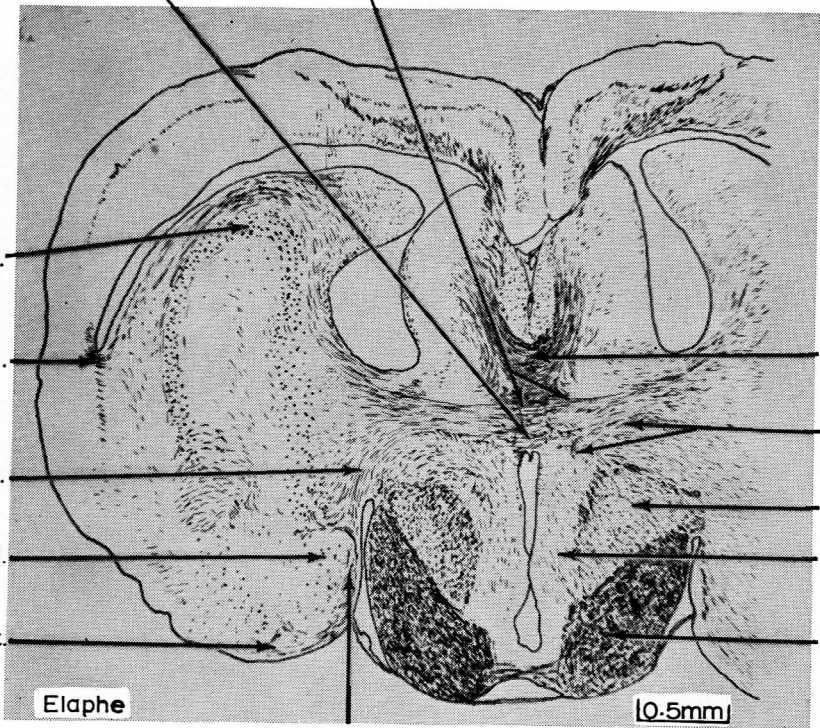
area preop.

tr. op.

Elaphe

0.5mm

lat. cort. hab. cmpt. st. med.



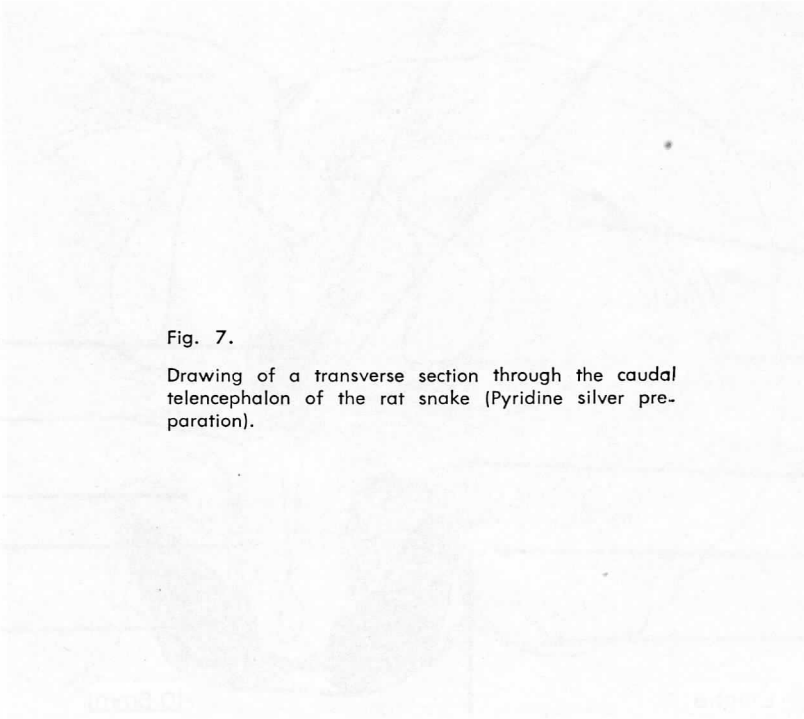


Fig. 7.

Drawing of a transverse section through the caudal telencephalon of the rat snake (Pyridine silver preparation).

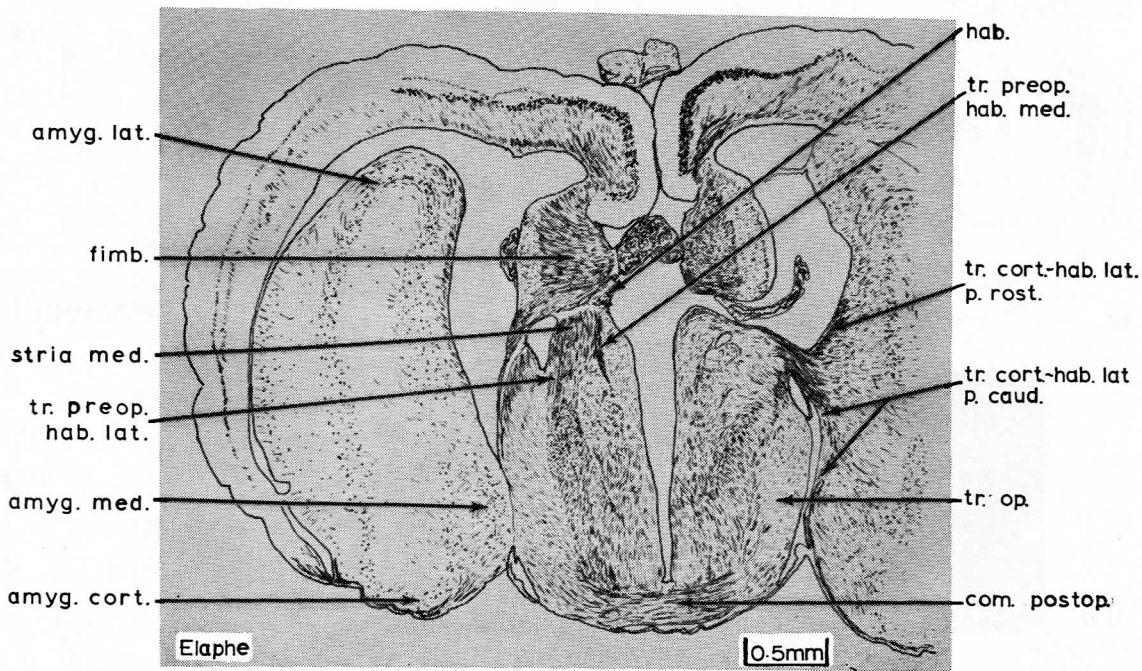
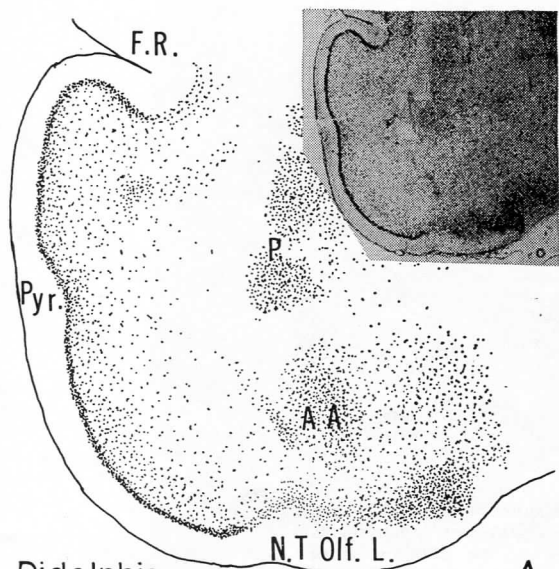


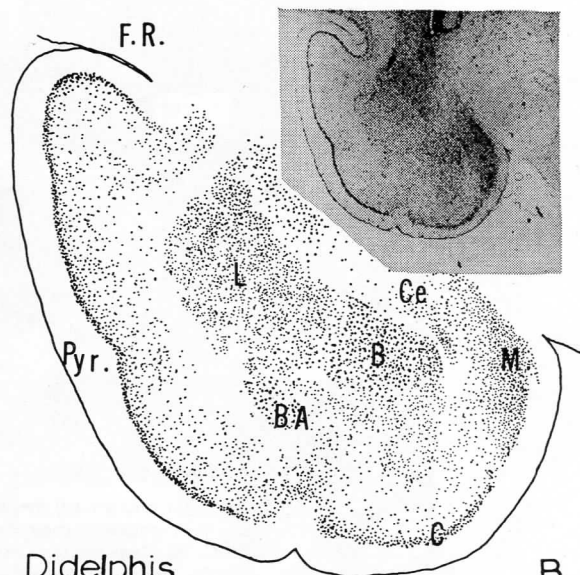
Fig. 8.

Cross sections through the amygdaloid complex of the opossum, drawn from Nissl preparations 25X. A) At the level of the anterior commissure, B) through the middle of the amygdala, C) through the caudal third of the amygdala, D) at the most caudal part of the amygdala. Each drawing is made from the photographic insert.



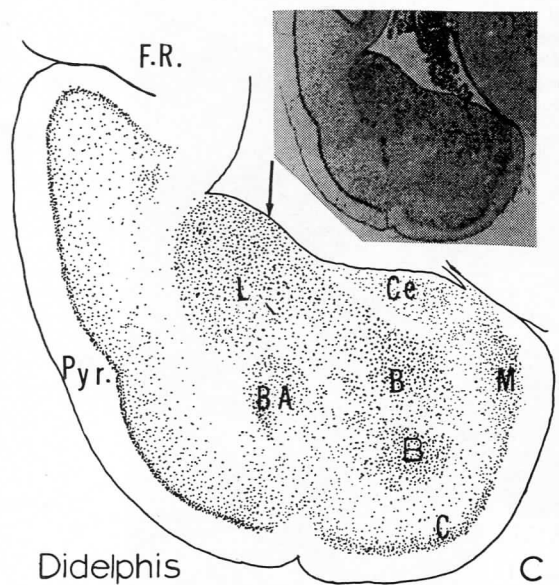
Didelphis

A



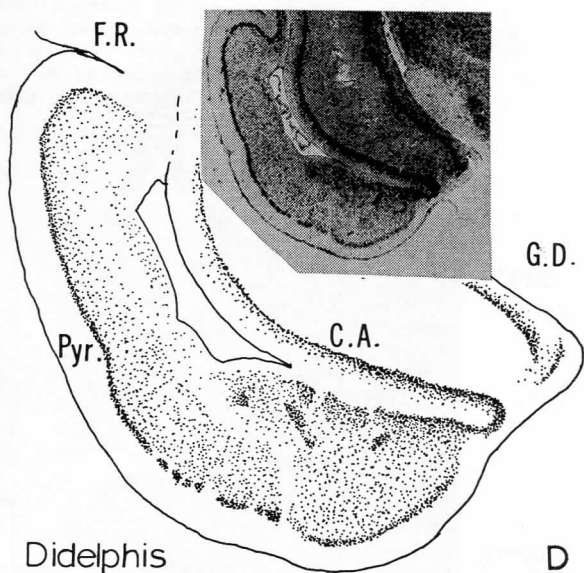
Didelphis

B



Didelphis

C



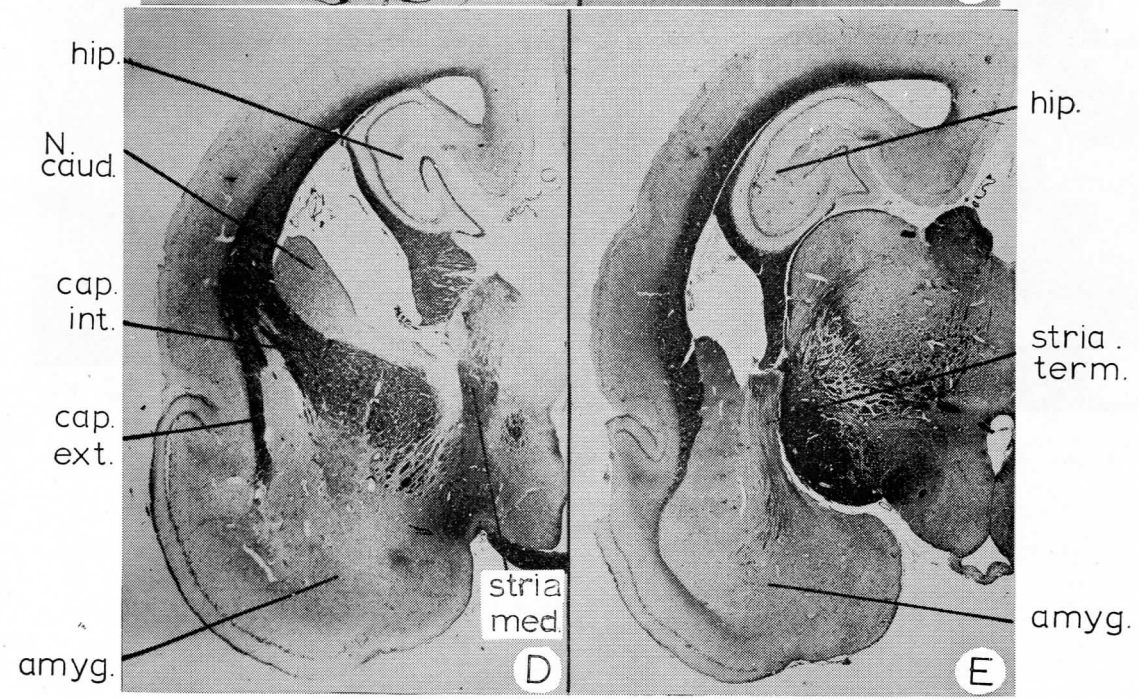
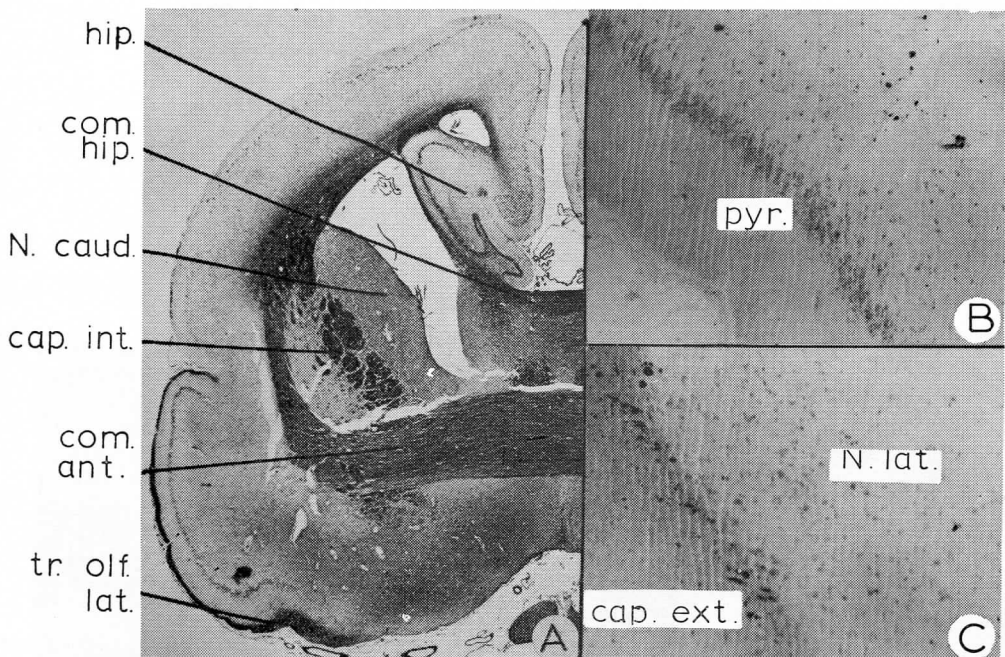
Didelphis

D



Fig. 9.

A) Cross section of the opossum brain at the level of the anterior commissure. Weigert preparations 25X. B) Degenerated fibers in the piriform cortex of the opossum. Marchi preparations 350X. C) Degenerated fibers in the external capsule and lateral amygdaloid nucleus. Marchi preparations 350X. D) Cross section through the opossum brain at the level of the stria medullaris. Weigert preparation 25X. E) Cross section of the opossum brain at the level of the origin of the stria terminalis. Weigert preparation 25X.



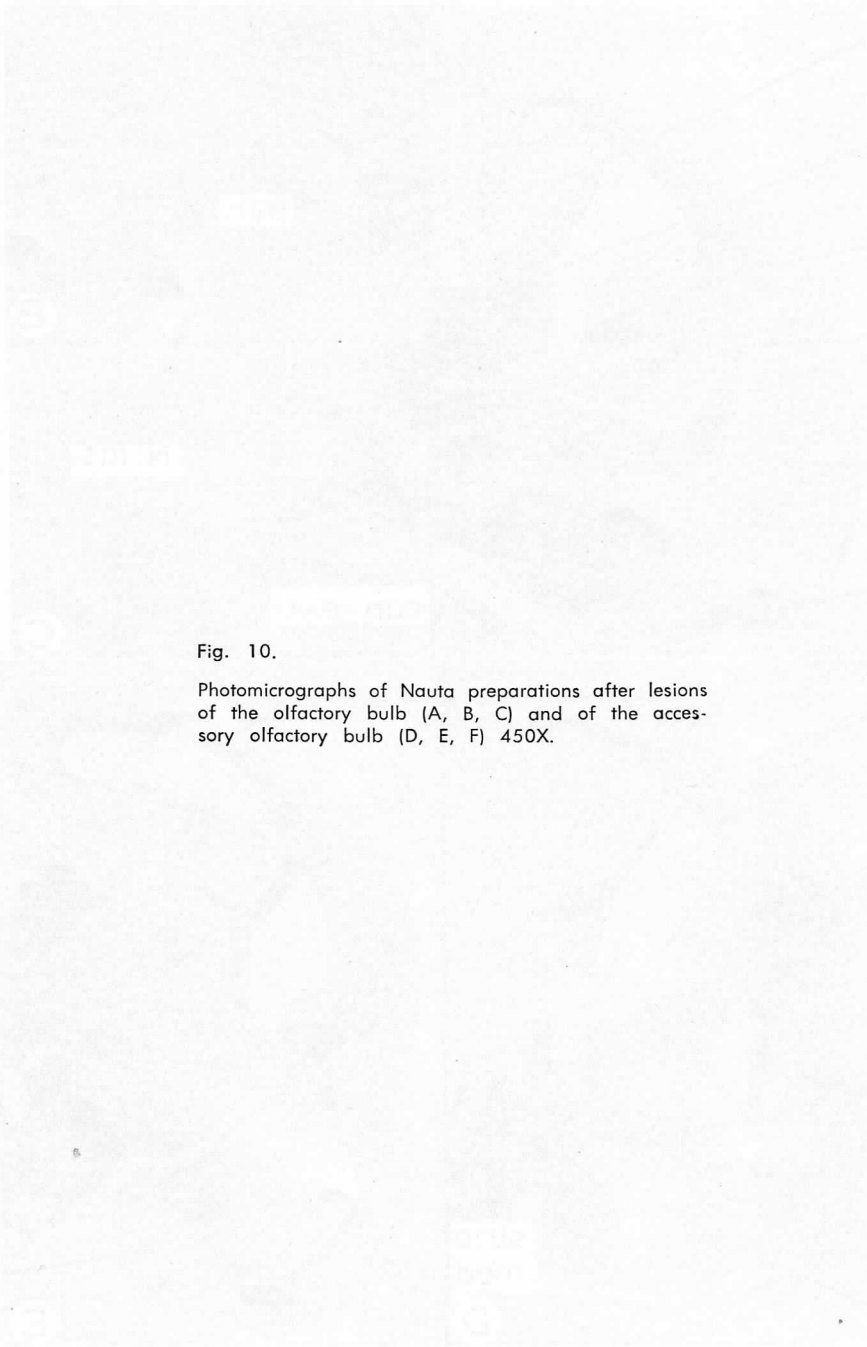
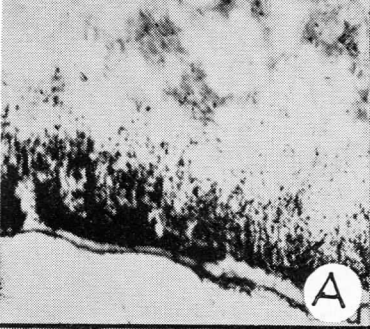


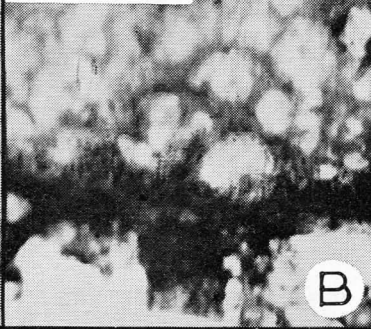
Fig. 10.

Photomicrographs of *Nautia* preparations after lesions of the olfactory bulb (A, B, C) and of the accessory olfactory bulb (D, E, F) 450X.

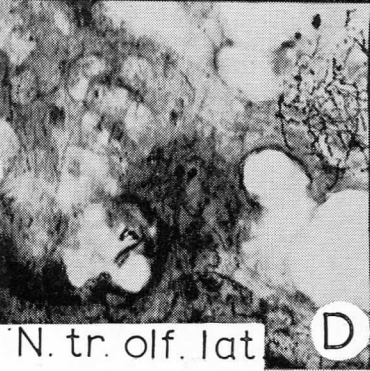
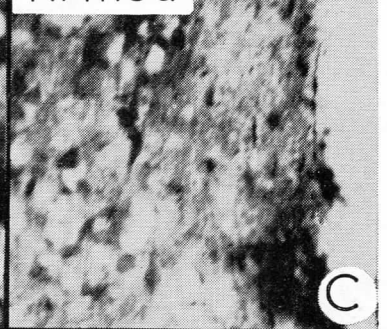
N. tr. olf. lat.



N. cort.

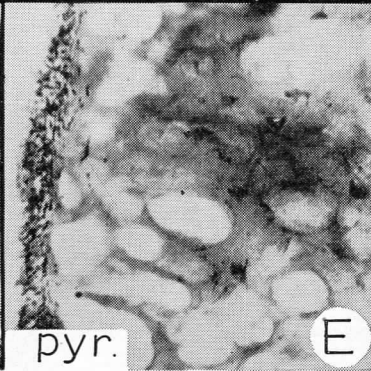


N. med



N. tr. olf. lat.

pyr.



N. lat.

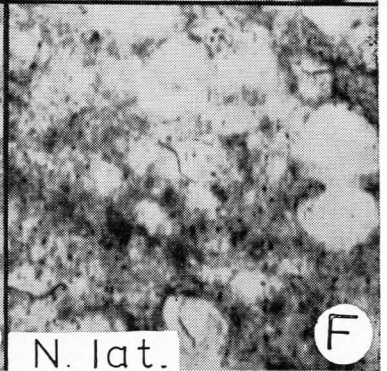


Fig. 11A.

High power photomicrograph of a nerve cell body in the basolateral nuclear group of the amygdala of the goldfish. (Golgi)

Fig. 11B.

High power photomicrograph of the nerve cell body in the corticomедial nuclear group of the amygdala of the goldfish. (Golgi) •

Fig. 11C.

High power photomicrograph of the nerve cell body. Basolateral amygdaloid nuclear group of the frog. (Golgi)

Fig. 11D.

High power photomicrograph of a nerve cell body. Corticomедial nuclear group of the frog. (Golgi)

Fig. 11E.

Transverse section through the amygdala of the goldfish. (Thionin)

Fig. 11F.

Transverse section through the amygdala of the frog. (Thionin)

N. basolat.

N. corticomed.

N. basolat.

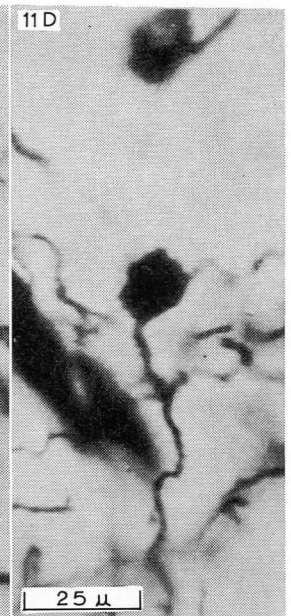
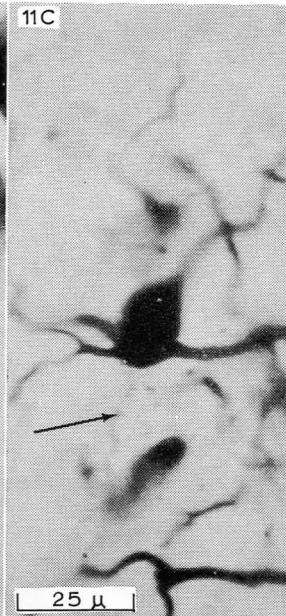
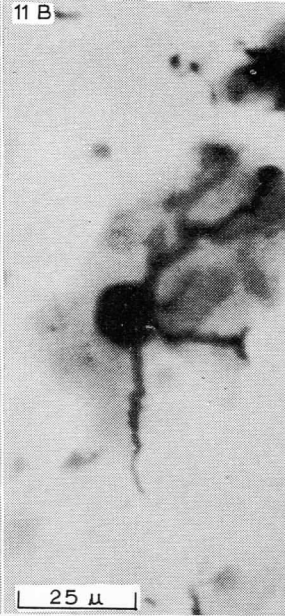
N. corticomed.

11A

11B

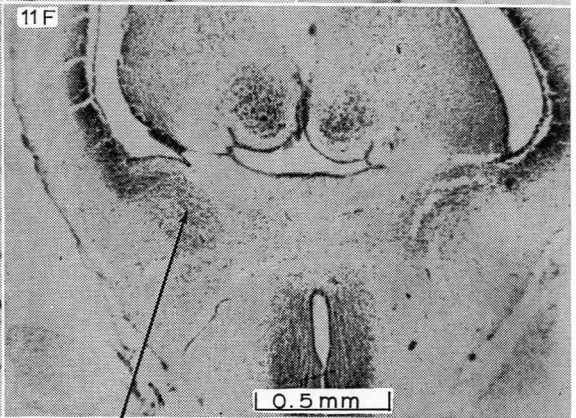
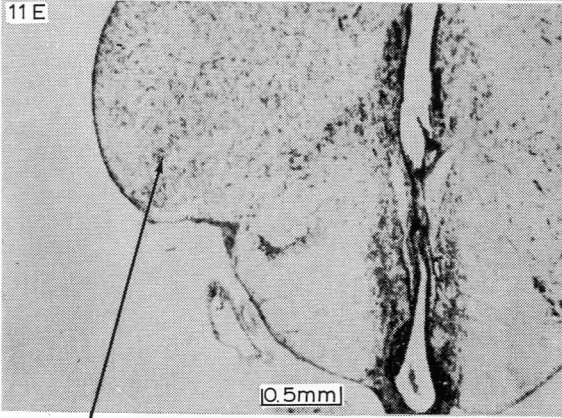
11C

11D



11E

11F



N. basolat.

C. auratus

N. basolat.

R. pipiens

Fig. 12A.

High power photomicrograph of a nerve cell body in the lateral amygdaloid nucleus of the turtle. (Golgi)

Fig. 12B.

High power photomicrograph of a nerve cell body in the medial amygdaloid nucleus of the turtle. (Golgi)

Fig. 12C.

High power photomicrograph of a nerve cell body in the lateral amygdaloid nucleus of the marmoset. (Golgi)

Fig. 12D.

High power photomicrograph of a nerve cell body in the medial amygdaloid nucleus of the marmoset. (Golgi)

Fig. 12E.

Transverse section through the amygdala of the turtle. (Thionin)

Fig. 12F.

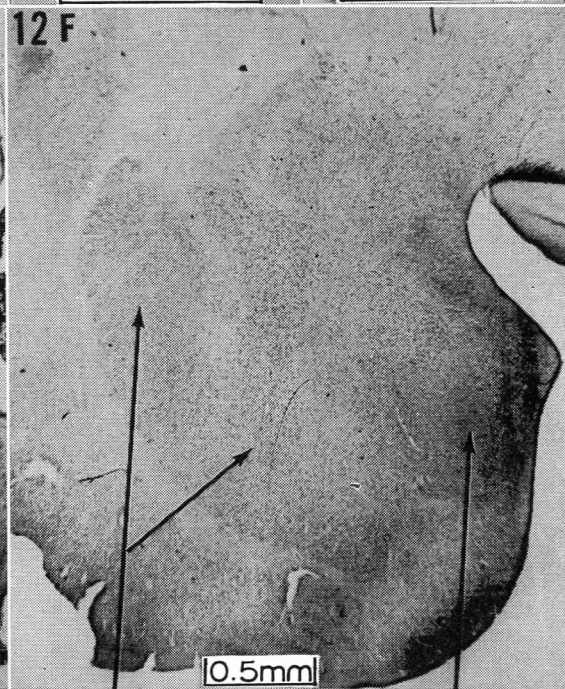
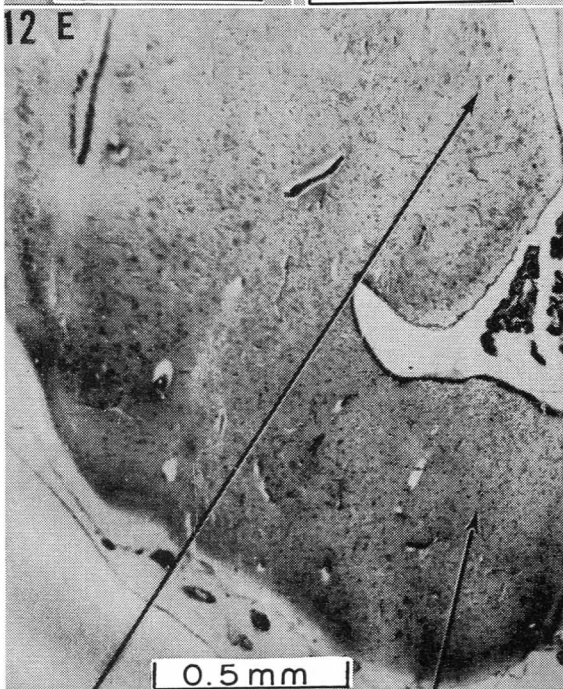
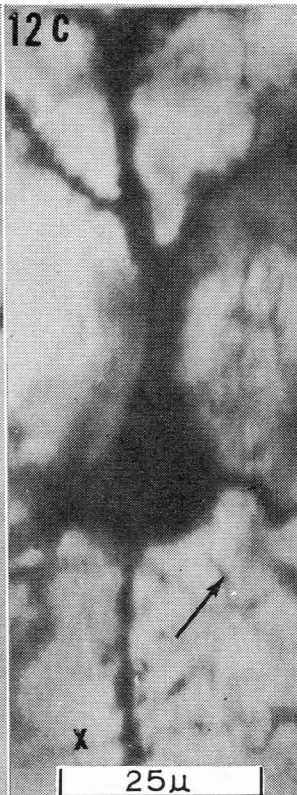
Transverse section through the amygdala of the marmoset. (Thionin)

N. basolat.

N. corticomed.

N. basolat.

N. corticomed.



N. basolat.

N. corticomed.

N. basolat.

N. corticomed.

Graptemys.

Hapale

Fig 13.

Schematic diagram illustrating the phylogenetic pattern of the fiber connections of the amygdala.

AMYGDALAR CONNECTIONS

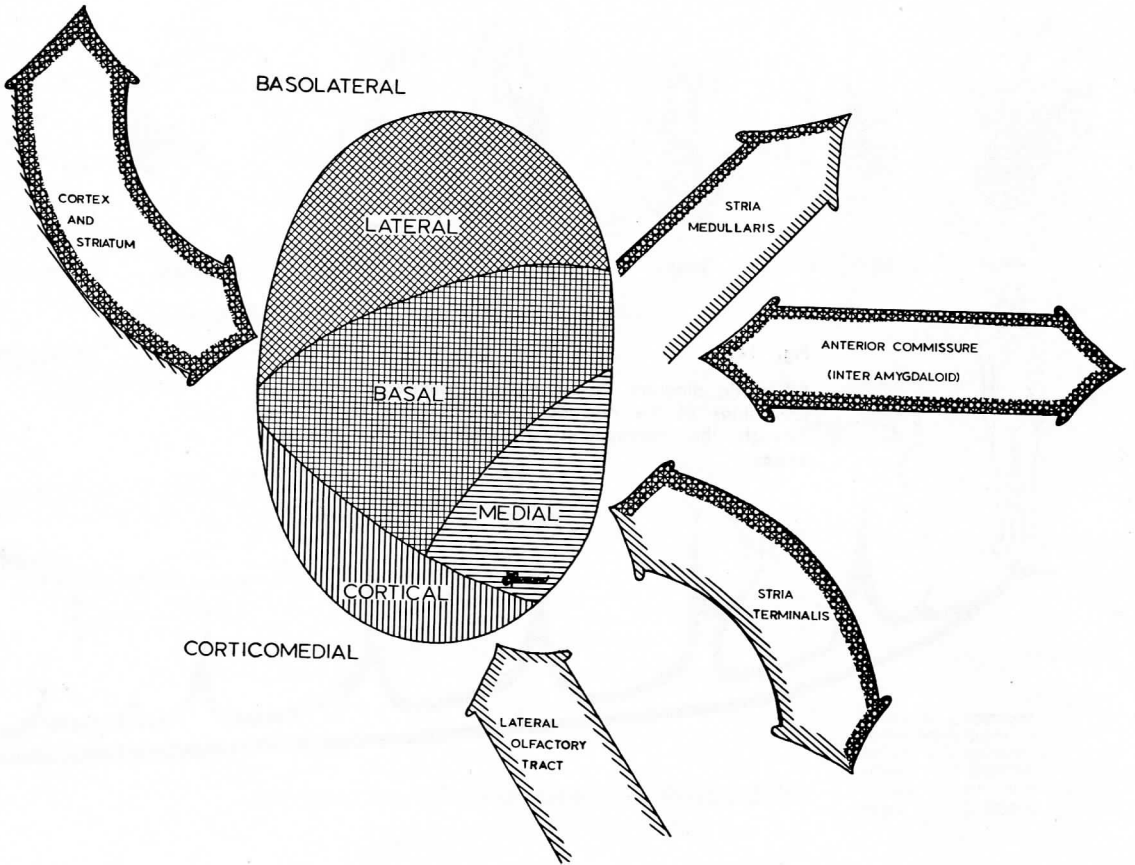
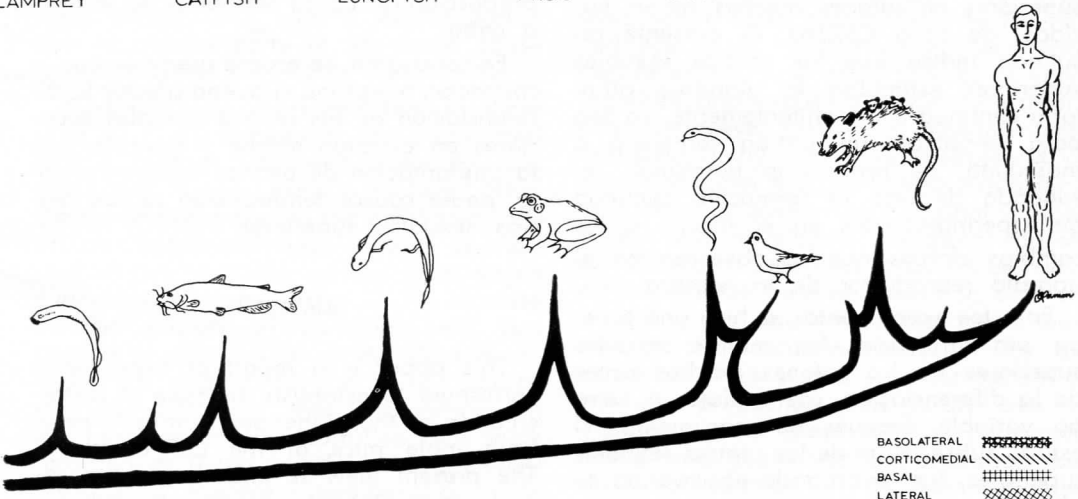
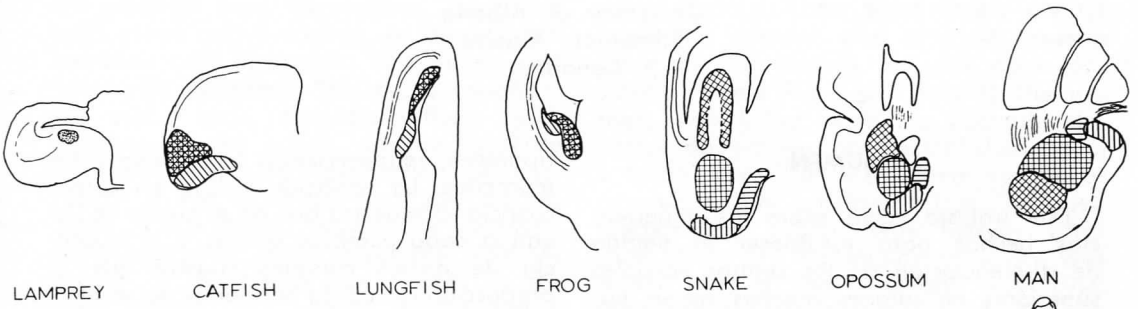


Fig. 14.

Schematic diagram illustrating the telencephalic relationships of the amygdala by transverse sections through the hemispheres of representative vertebrates.

Higher α -x centers in newborn mice



AMYGDALAR RELATIONSHIPS IN PHYLOGENY

BASOLATERAL	
CORTICOMEDIAL	
BASAL	
LATERAL	
CORTICAL	
MEDIAL	

Higher sex centres in newborn male mice

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Edmonton, Alberta
Canada.

RESUMEN

Este trabajo versa sobre las experiencias hechas para establecer el tiempo de diferenciación de los centros sexuales superiores en ratones machos recién nacidos, de cepa C57/6J. El presente resultado indica que los centros sexuales superiores estimulan la glándula pituitaria continua o intermitentemente, ya sea para establecer un patrón femenino o masculino, de producción hormonal, resultando de esto la formación continua de espermatozoides en el macho o los cambios cíclicos que se observan en el aparato reproductor de la hembra.

En estos experimentos se hizo una prueba para influenciar los centros sexuales superiores de los ratones machos antes de la diferenciación, castrándolos a tiempo variable después del nacimiento. El estado subsecuente de los centros sexuales superiores, fue investigado observando su efecto a través de la pituitaria en ovarios que fueron implantados a estos ratones. Dichos ovarios se estudiaron observando su efecto en vaginas también implantadas. Se hicieron exámenes diarios de las células descamadas de la vagina. En el adulto, hembra normal, la descamación de las células epiteliales cornificadas en el exudado vaginal es intermitente y coincide con la ovulación y la fase estrogénica. Después las células cornificadas des-

aparecen, reapareciendo la mucosa y los leucocitos. La variación cíclica, en consecuencia, demuestra que en el ovario se llevan a cabo cambios cíclicos y la ausencia de dichos cambios sugiere que la producción cíclica de hormonas no se lleve a cabo.

En conclusión, se espera asegurar que la castración temprana sí pueda afectar la diferenciación de los centros sexuales superiores en el ratón macho y también que la implantación de ovarios tempranamente, podía causar feminización de los centros sexuales superiores.

SUMMARY

This paper is a report of experiments performed to establish the time of differentiation of the higher sex centres in newborn male mice of the C57/6J strain. The present view is that the higher sex centres stimulate the pituitary in either a continuous or an intermittent manner to establish either a male or a female pattern of hormone production resulting either in the continuous production of spermatozoa in the male or in the cyclic changes in the female reproductive system. In these experiments an attempt was made to influence the higher sex centres in male mice before differentiation by castrating them at varying times after

birth. The subsequent state of the higher sex centres was investigated by observing their effect through the pituitary on implanted ovaries. The ovaries in turn were studied by observing their effect on vaginas implanted into the male mice by daily examination of the exfoliated cells from the vaginas. In the normal adult female mouse there is an intermittent desquamation of cornified epithelial cells in the exudate from the vagina, coincident with estrus and ovulation. At diestrus the cornified cells disappear, and mucus and leucocytes reappear. This cyclic variation, the estrus cycle, therefore reflects cyclic changes in the ovary, and its absence suggests that cyclic production of hormones is not occurring. By these means, then, it was hoped to ascertain whether early castration could affect the differentiation of the higher sex centres in the male mouse, and also if the implantation of ovaries early in life could cause feminization of the higher sex centres.

Firstly, it was confirmed that in these mice an ovary implanted into a castrated female mouse functioned normally and produced regular estrous cycling. Next, the correlation between the exfoliated cells of an implanted vagina and a normal vagina with the cycling of a single ovary was confirmed (Fig. 1). An increase in the number of cornified cells appeared at intervals of a few days in an implanted vagina on the same day or the day following their appearance in the host vagina. The cornified cells were recorded as 0 (no cornified cells), 1 (very few), 2 (a moderate number), or 3 (a large number). Cornified cells persisted longer in the vaginal implants than in the host vagina, but nevertheless there was a definite cyclic variation. If the host vagina underwent a period of anestrus or constant estrus, then the implant showed a corresponding reduction or increase in the number of cornified cells. It is therefore confirmed that the implanted vagina reflects ovarian cycling but not so accurately as the host's own vagina.

32 male mice were castrated at known times from four days after birth up to 18 months after birth. At the time of operation they received one ovary from a sibling subcutaneously on their anterior abdominal wall. When the female siblings were mature their vaginas were implanted into the male mice and daily smears were taken start about ten days later. After a few days a definite pattern became obvious. There were nearly always leucocytes present, and in most cases a large number of cornified cells were constantly present (Fig. 2 and Plate 1). The animals were killed when the vaginal exfoliation pattern was well established about a month later. This is a pattern sometimes referred to as constant estrus. At post-mortem the vagina was heavily cornified (Plate II). The implanted ovary was larger than a normal ovary, with cysts which were often hemorrhagic to the naked eye (Plate III). Histological examination revealed large cystic follicles of varying sizes, and interstitial tissue. No corpora lutea were ever seen. This experiment established firstly that mice castrated 4 or more days after birth all behaved in a similar fashion on ovarian and vaginal implantation and developed persistent vaginal cornification. Secondly, after three days after birth the time of castration of male mice has no relation to the future pattern of sex hormone production by the pituitary, which was a constant pattern of hormone production, and thirdly, ovarian implantation in these mice is performed easily and the graft is functional, but does not function in the same manner as in the female.

13 male mice were castrated at known times up to three days after birth. Two of these received successful ovarian implants at that time. Five had unsuccessful ovarian implants at the time of castration but were re-implanted with ovaries at three months. Six more received ovarian implants at two months. All received vaginal implants at two months. Cornification occurred in the vaginal smears, but at a lower level

than in mice castrated four or more days after birth (Fig. 3 and Plate IV). The vaginal implants were less cornified (Plate V) and there were areas where there were mucous cells (Plate VI). The ovaries were small with occasional large hemorrhagic follicles (Plate VII). Interstitial tissue was present. There was luteinisation at the edge of some of the follicles which was not seen in the mice castrated 4 or more days after birth (Plate VIII). No corpora lutea were ever seen. The time at which the ovary was implanted did not seem to affect the subsequent behaviour of the mice whereas the times of castration appeared critical. There was never any evidence of ovulation, but the ovaries appeared to produce estrogen, as reflected by the daily vaginal smears, to a less degree than in those mice castrated 4 or more days after birth.

After this work was begun, Yasaki¹ reported that he implanted ovaries into newborn castrated male rats. He considered that implanted vaginas in close proximity to the ovaries reflected cyclic changes in the ovaries in some cases. He concluded that the hypothalamic centres in male rats were not differentiated fully until 3 days after birth. Up to this time the future male pattern of hormone production could be converted into the female pattern by replacing the testes with an ovary. The experiments reported here have not managed to confirm this for mice.

Harris and Levine² have reported recently on related work in rats. They gave estradiol to male rats when four days old and found that the subsequent male pattern of behaviour became disrupted. They felt that the estradiol affected the developing testes so that a partial physiological castration occurred. They found little evidence that female cyclic pattern in males treated with estrogen at four days of life. They concluded that newborn rats possess a sexually undifferentiated brain. During development the higher sex centres become organised in the female or male pattern

depending on whether the male sex hormone is present or absent.

In the experiments reported here the presence of a constantly heavily cornified smear, and the results of postmortem examination suggest that ovaries implanted into male mice castrated four or more days after birth are subject to stimulation to produce estrogen at a constant rate and not in an intermittent manner as in the female. The future male pattern of constant hormone production is therefore, firmly established by four days after birth. In mice castrated up to three days after birth there is apparently a lower level of estrogen production on subsequent ovarian implantation. It is concluded that in mice castrated shortly after birth and which received an ovarian implant the male pattern of hormone production develops but to a lesser degree than in mice castrated when older. So far, no vaginal cycling has taken place in any of the mice castrated just after birth.

In the male, then, at the time of birth, testicular secretion has been present long enough to start the differentiation towards the male type of higher sex centre and to prevent the development of the ability to stimulate the greater FSH production of the female. The removal of the testes between birth and three days after birth can only stop further differentiation. By 4 days after birth differentiation is complete so removal of the testes after that time can have no effect. The substitution of the testes by an ovary after birth does not influence the higher sex centres which already have been under the influence of testicular secretion.

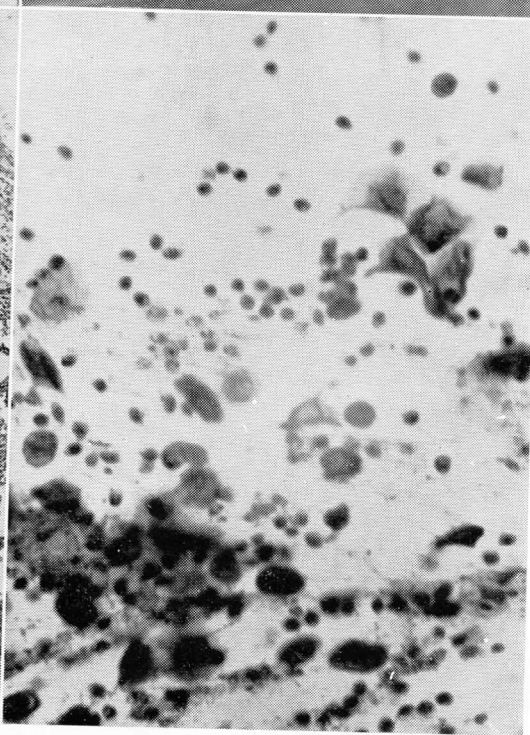
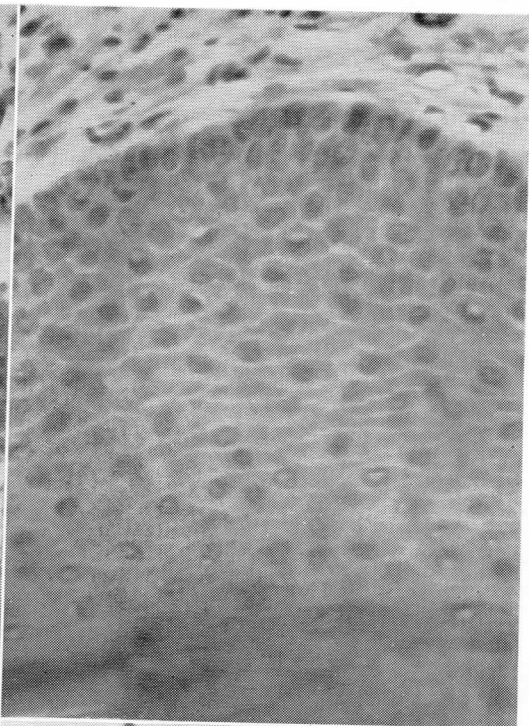
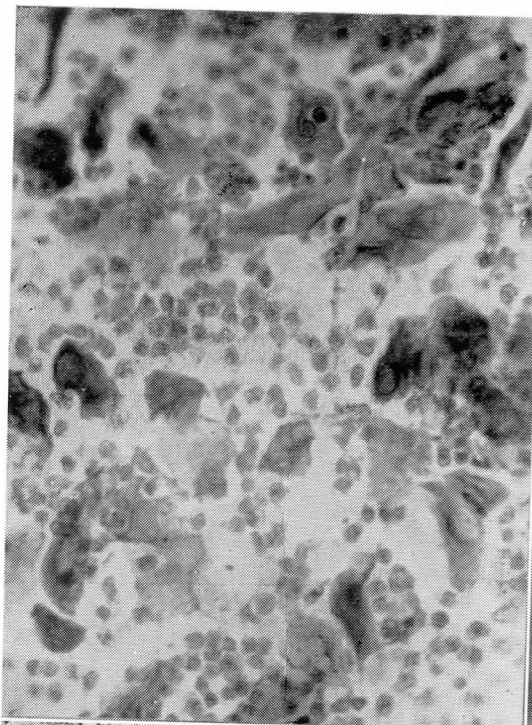
The conclusions are firstly that the higher sex centres are differentiated at birth to such a degree so as to make it impossible to convert them into the female type of higher sex centre. Secondly, removal of the testes, in the first few days after birth, results in a less positive pattern of hormone production than in mice castrated at a later date. Thirdly, by four days after birth, the relationship between

the higher sex centres, the pituitary and the gonad is firmly established in the male pattern in male mice. Therefore, the higher sex centres in male mice are not differentiated completely until four days after birth in these C57 Black mice.

References:

- ¹ Yasaki, I., Annot. Zool. Japon. 33: 217. 1960.
- ² Harris, G. W. and S. Levine. J. Physiol. 181: 379. 1965.

- I. Smear from vagina implanted into a male mouse which was castrated and received an ovarian implant more than three days after birth, showing heavy cornification of epithelial cells. Hematoxylin and Eosin X 365.
- II. Vaginal implant from male mouse which was castrated and received an ovarian implant more than three days after birth, showing heavy cornification and thick epithelium. Hand and interstitial tissue. Hand E X 58.
- III. Ovary from male mouse which was castrated and received an ovarian implant more than three days after birth, showing cystic follicles, and interstitial tissue. H and E X 58.
- IV. Smear from vagina implanted into a male mouse which was castrated and received an ovarian implant less than three days after birth, showing some cornified cells, some non-cornified epithelial cells, and many leucocytes. Hand E X 365.



- V. Vaginal implant from male mouse which was castrated less than three days after birth and received an ovarian implant at 2 months of age, showing cornification, but thinner epithelium than in II, Hand E X 365.
- VI. A different area of section used in V, showing mucification of outer layers of vaginal epithelium. Hand E X 365.
- VII. Ovary from male mouse which was castrated less than three days after birth and received an ovary at 3 months, showing one follicle and interstitial tissue. Note small size of ovary. Hand E X 58.
- VIII. Ovary from male mouse which was castrated less than three days after birth and received an ovary at two months, showing the edge of a follicle which is partially luteinised Hand E X 365.

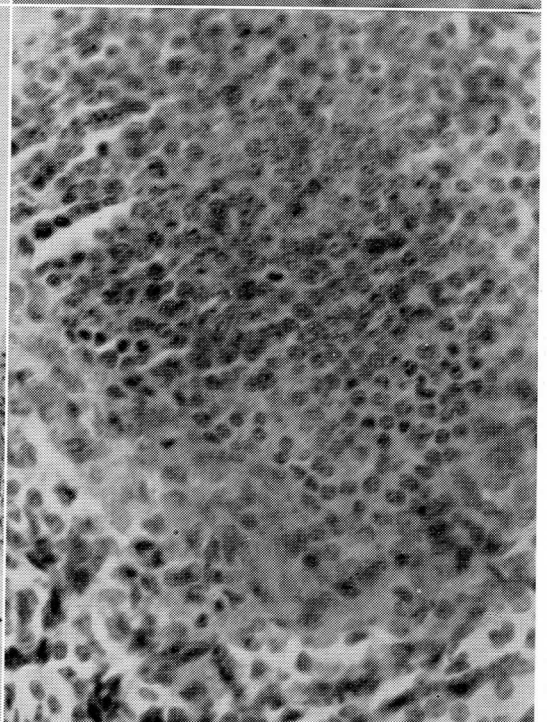
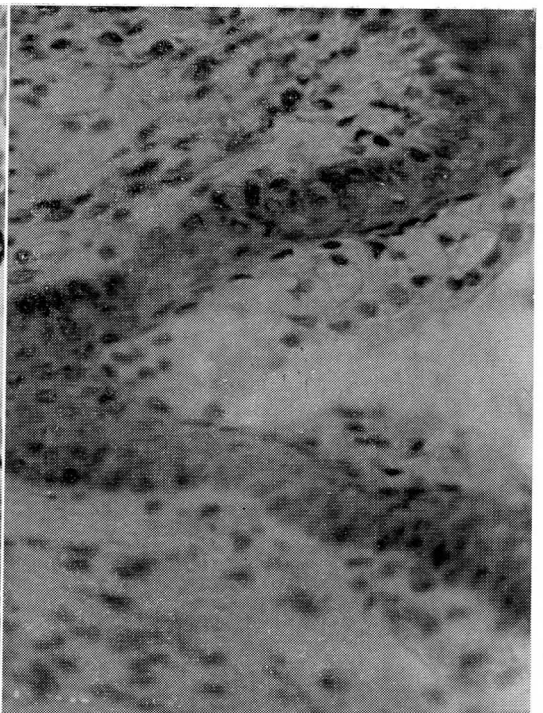
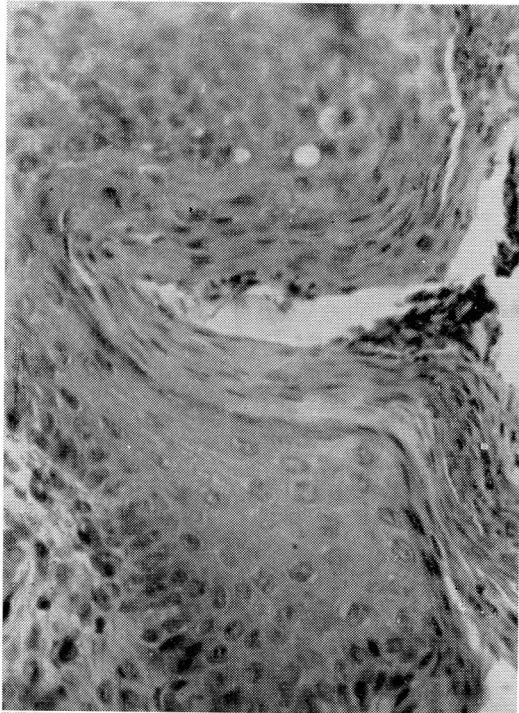


Figure 1.

Vaginal smears of female mice with one ovary which received additional vaginal implants.

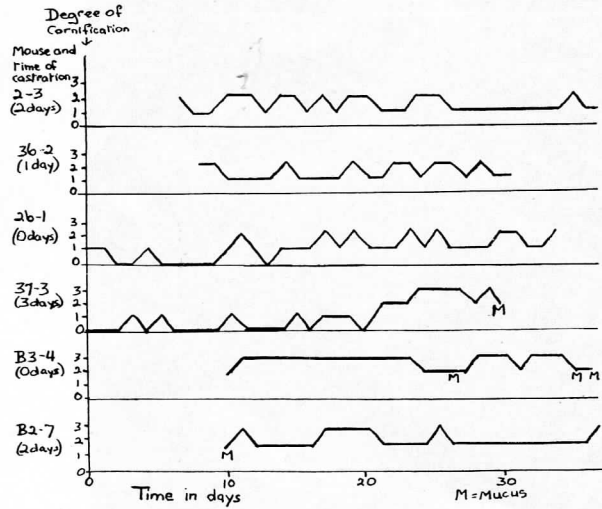
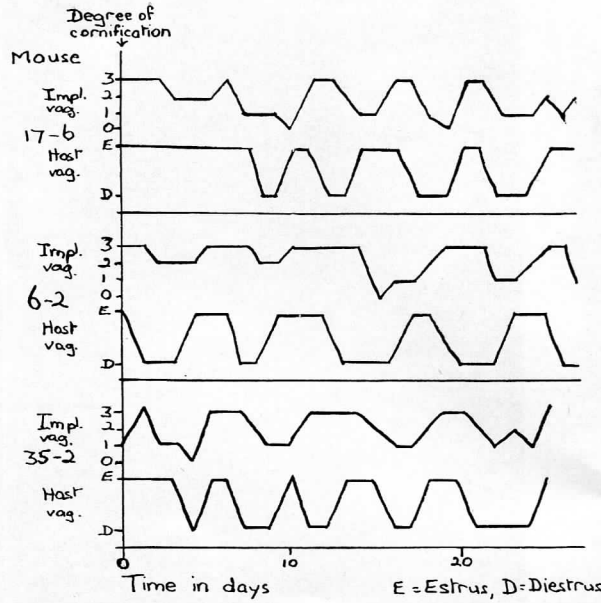


Figure 2.

Vaginal smears from male mice which were castrated and received ovarian implants four or more days after birth and received ovarian implants at a later date.

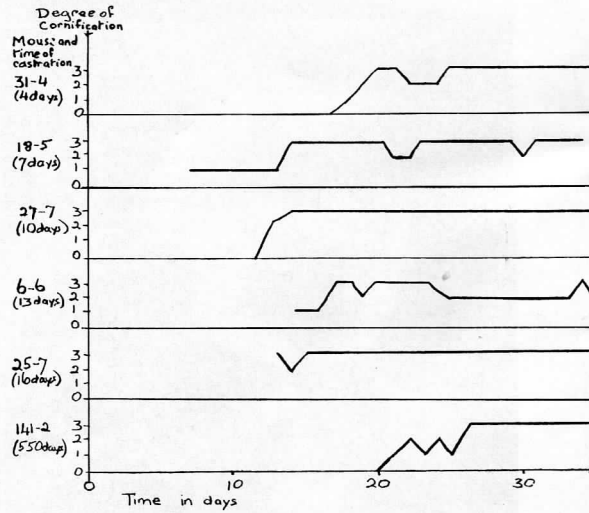


Figure 3.

Vaginal smears from mice castrated up to three days after birth which received ovarian implants at that time, three months later or two months later. Vaginas implanted at two months.

COLLABORATOR'S INFORMATION

"Archivos Mexicanos de Anatomía" appears every four months as an official organ of the Mexican Society of Anatomy. Besides general information, it also contains articles on cordate, descriptive or experimental anatomy; methods, technics and abstracts of articles from morphological magazine.

On original publication it is our wish to present articles no exceeding twenty pages and with the following qualification: Abstract of 200 words or less, to be published ahead of the introductory text in place of a summary at the end; and written with the purpose of informing the significant content and conclusion of the article and not as a mere description.

Manuscripts and drawings should be sent by registered post paid to the Editorial Commission formed by Doctors Salvador Gómez Alvarez and Fernando Quiroz Pavia, Apartado Postal 25279 Admon. de Correos 70. Manuscripts should be typewritten in double spacing on one side only of a letter-size sheet and should be mailed between two cardboards to avoid folding.

Send the work, original drawings, carbon copy and photostat of the illustrations. The authors should indicate on the article, the adequate position of the figures.

Texts and drawings should have the complete address of the author. The Editorial Staff reserves its rights to return to the author to be checked, the writings and illustrations approved which are not in the correct form according to the printer.

When it is decided that the written or illustrated material is excessive or too costly, authors may be requested to play the excess cost.

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En trabajos originales nuestro deseo es publicar artículos que no excedan de 20 páginas y que llenen los siguientes requisitos:

Resumen de 200 palabras o menos, para ser publicado antes de la Introducción en lugar del sumario que se pone al final; y escrito con el objeto de informar a los lectores del significado del contenido y conclusiones del artículo y no como una simple descripción.

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Enviar el trabajo y dibujos originales e igualmente una copia al carbón y fotocopias de las ilustraciones. Los autores deberán indicar en el artículo, la posición adecuada de las figuras.

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