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A
CARMELITA
COCO Y
LALO

A MIS SINODALES,
AMIGOS Y COLABORADORES

MI PERENNE AGRADECIMIENTO A LOS DRS. CARLOS GUZMAN-FLORES Y
MANUEL ALCARAZ VERDUZCO POR SUS ENSEÑANZAS

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RESUMEN

En este estudio, se hace una breve revisión de los conceptos básicos relacionados con la Neurobiología del Desarrollo, y de los hallázgos más relevantes obtenidos por nuestro grupo de investigación en esta área. El tejido cerebral en desarrollo, se ensambla y organiza por la influencia de factores genéticos programados, en asociación con diversos factores perinatales no programados como la privación parcial de alimento, los niveles plasmáticos de hormonas y fármacos en el medio interno y diversas rutinas de privación o de estimulación sensorial. En esta área del conocimiento se emplea comúnmente a los roedores como modelo experimental, ya que éstos al igual que el hombre, presentan un desarrollo de tipo altricial. Para la evaluación de los efectos causados por diferentes influencias no programadas, se emplea frecuentemente el enfoque experimental multidisciplinario, en el cual se combinan el análisis citoarquitectónico fino de áreas específicas del cerebro,. con sus correlatos neurofisiológicos, neuroquímicos y conductuales. A lo largo de este trabajo y a manera de ejemplo, se consignan algunas de las alteraciones morfológicas, conductuales y electrofisiológicas provocadas por la administración neonatal de hormonas neurotrópicas como la T_4 y los corticoides suprarrenales. Estas hormonas al acelerar o retardar respectivamente el desarrollo cerebral, nos han permitido utilizarlas como herramientas para interferir con los procesos ontogenéticos del desarrollo neural y de este modo, analizar los cambios que se dan en la plasticidad cerebral bajo esas circunstancias. Asimismo, los modelos experimentales que hemos desarrollado para su evaluación, por su confiabilidad se siguen empleando para analizar otras influencias, o para probar nuevas hipótesis. La identificación de las acciones hormonales también ha sido de gran utilidad, para generar nueva información acerca de los mecanismos de regulación de la ontogenia cerebral y su relación con el desarrollo de la función neural en el hombre. Nuestro grupo ha hecho contribuciones relativas a cómo la privación parcial diaria de alimento durante la etapa crítica del desarrollo cerebral, deteriora

el crecimiento de los árboles dendríticos de poblaciones neuronales que participan en el control de la información sensorial. En efecto la corteza cerebral, la amígdala temporal, los núcleos talámicos, la substancia gris central y el complejo olivar superior, muestran una reducción significativa en el crecimiento dendrítico, lo cual sugiere que los procesos de recepción, integración y elaboración de respuestas neuronales están seriamente alterados. Así, hemos contribuido a definir la existencia de un substrato neuronal permanentemente dañado, que va asociado usualmente a una función cerebral deteriorada. Nuestros estudios en el modelo de la rata, nos han permitido indagar acerca de los mecanismos básicos a través de los cuales podría generarse el retardo mental asociado a las alteraciones hormonales perinatales, a la desnutrición y a la reducción de estímulos sensoriales. Asimismo, que mediante la rehabilitación nutricional o bien del empleo de diversas rutinas de estimulación sensorial durante el periodo neonatal, es posible revertir algunos efectos deletéreos provocados por la desnutrición perinatal sobre la arquitectura y la función cerebrales. Nuestro grupo ha correlacionado las alteraciones en la citoarquitectura cerebral asociadas a la desnutrición neonatal, con los efectos a largo plazo sobre distintos patrones conductuales. Particularmente hemos comprobado que la conducta de autoaseo, se encuentra sistemáticamente incrementada, posiblemente por el daño causado a los mecanismos cerebrales que participan en su regulación. De la misma manera que la capacidad exploratoria, la habituación, la atención selectiva y la conducta maternal, se reducen significativamente en animales adultos bién nutridos, que fueron desnutridos en etapas tempranas de su desarrollo neonatal. Estas alteraciones adquieren gran significación, porque dan evidencia directa de la reducida capacidad cerebral del individuo desnutrido durante la etapa neonatal, para interaccionar con el medio ambiente, para el aprendizaje y para la deficiente atención maternal hacia las crías recién nacidas.

En los últimos años, nuestro grupo ha estudiado los efectos producidos por la desnutrición neonatal sobre el desarrollo morfológico, electrofisiológico y

conductual de la rata. Algunos de nuestros hallazgos han sido pioneros en este campo y han servido de guía para generar nuevo conocimiento a partir de los conceptos que hemos establecido. Asimismo, otros han sido novedosos por las metodologías relativamente sencillas que hemos utilizado para evaluar la función cerebral deteriorada por la desnutrición perinatal.

ABSTRACT

During neural ontogeny the cytoarchitectonic arrangement of various brain structures are primarily dependent on genetic influences in association with nonprogrammed epigenetic factors. In this regard it is generally accepted that the events in the internal and external environments are continuously providing a stream of information that pours through the sense organs into the brain. Thus, evidence indicating the fundamental role of hormones or other chemical compounds during early stages of neural development, has been reported. It is also known that external environmental influences like handling and nutrition during early life are also basic factors underlying the maturation of the nervous system and which will determine its future functional pluripotential flexibility. To evaluate the effects of these influences a multidisciplinary approach is commonly employed, including the fine cytoarchitectonic analysis of different brain areas in association with their electrophysiological, neurochemical and behavioral correlates. In the present study I will discuss those relevant findings generated by our research group in the Universidad Nacional Autónoma de México that have been contributing to the knowledge of brain ontogeny in the rat. Particularly, how neonatal undernutrition interferes with brain growing and its function by using different experimental models. The data here presented provide information that early undernutrition is one of the external environmental factors which, interacting with the growing neurons affects the neural substrate maturation, the capacity to generate electrical activity and the neural mechanisms underlying brain plasticity. These deleterious effects could impede the newborn in receiving and using novel environmental cues for neuronal growth and in the acquisition of new cognitive processes. Present findings provide also evidence of how altered brain processes following neonatal undernutrition can be ameliorated by exposing the newborns to different early dietary and sensorial rehabilitation procedures.

INTRODUCCION

En las últimas 5 décadas, se han generado numerosas contribuciones acerca de los mecanismos que controlan el desarrollo postnatal del cerebro en los mamíferos, y sobre las posibles implicaciones que éstos tienen para explicar los cambios funcionales en el corto y en el largo plazo. Asimismo, poco se ha hecho con respecto a la evaluación de las influencias ambientales que afectan el curso del desarrollo cerebral en el periodo previo al destete. Un factor del medio ambiente externo que contribuye importantemente al desarrollo postnatal, es el aporte de nutrimentos a los tejidos en fase de crecimiento, que puede tener una gran significación para entender las alteraciones funcionales vistas en el individuo adulto desnutrido durante la etapa perinatal.

En el animal maduro los cambios de funcionalidad cerebral en respuesta a las continuas demandas de los estímulos ambientales, son realizables sobre la base de la existencia de fenómenos plásticos en el sistema nervioso central (SNC), que adquieren su máxima expresión durante esta etapa de la vida. En el cerebro inmaduro por el contrario, conforme los procesos de neurogénesis, migración, diferenciación, crecimiento y conectividad van sucediéndose en el tiempo, se va adquiriendo gradualmente el substrato nervioso del que dependerá la plasticidad cerebral futura.

Los hallazgos previos de nuestro laboratorio, sobre los efectos a largo plazo provocados por la privación neonatal de alimento en la rata, nos han permitido avanzar en la hipótesis de que la desnutrición neonatal, pudiera interferir en mayor grado con el crecimiento, conectividad y funcionalidad de sistemas polisensoriales en el SNC que poseen un alto grado de plasticidad, en comparación con los efectos sobre los sistemas constituidos por escaso número de neuronas. Esta hipótesis parece tener apoyo tanto en los resultados que hemos obtenido con la evaluación de los componentes de la conducta refleja, como con la deficiencia en los procesos nerviosos de gran complejidad, del tipo del aprendizaje, la emotividad, la atención

selectiva y la capacidad exploratoria, que se han observado en el animal adulto desnutrido durante la infancia.

En el presente trabajo, me permitiré realizar una breve revisión acerca del impacto que tienen algunos factores epigenéticos del tipo de las hormonas, el aporte de nutrimentos y la estimulación sensorial, sobre el desarrollo cerebral, destacando cuando sea pertinente algunos de los hallazgos experimentales sobre la ontogenia neural, con los que nuestro grupo de investigación ha contribuido al conocimiento de esta área.

El análisis formal de la ontogenia del SNC en los mamíferos, data de finales del siglo pasado cuando por primera vez se llevaron a cabo estudios sistematizados del desarrollo postnatal de la conducta (Small, 1899). En el comienzo de este siglo con el surgimiento de las técnicas morfológicas se llegó al conocimiento de que durante los periodos prenatal y postnatal, ocurren cambios progresivos en la organización citoarquitectónica de las estructuras cerebrales y su funcionamiento. Asimismo, se estableció que las neuronas siguen patrones secuenciales de desarrollo y conectividad, que son típicos de cada especie y modalidad sensorial (Cajal, 1899 a, b, 1900; Sugita, 1918; Lorente de Nó, 1941;Eayrs y Horn, 1955). En los últimos años con el advenimiento de las técnicas electrofisiológicas y bioquímicas, se ha dado también un gran avance en este campo, al saberse de la existencia de cambios funcionales dependientes de poblaciones reducidas de neuronas y de cómo éstos contribuyen a la maduración neuronal.

Resultado de todos estos estudios ha sido la acumulación de un gran número de evidencias experimentales relacionadas con los procesos de maduración estructural y funcional del SNC, con la ontogenia de su composición bioquímica, con el desarrollo del repertorio conductual del infante y la capacidad del organismo para responder y aprender tanto en situaciones naturales como experimentales (Ver, Himwich y Himwich, 1964). Asimismo, han surgido una serie de conceptos básicos que incluyen a los siguientes: 1) La relativa complejidad de los mecanismos

cerebrales durante etapas tempranas del desarrollo, permite un análisis más claro y simple de la secuencia con que las diferentes partes del SNC maduran e intervienen progresivamente en la función. 2) Se facilita el estudio sucesivo de las relaciones entre varias estructuras cerebrales, que son la base morfológica de procesos homeostáticos y conductuales a través de todo el desarrollo ontogenético. 3) Es posible aplicar estos estudios al conocimiento de las causas que modulan o dañan el crecimiento del SNC en el hombre. El SNC del infante es muy vulnerable durante el período perinatal, lo cual es causa de una alta tasa de deficiencia mental y de mortalidad infantil. Esta problemática pudiera quizás atenuarse o corregirse por el empleo adecuado de los conocimientos generados acerca de los procesos de la maduración neuronal. 4) Finalmente porque según estudios recientes, la interacción entre el organismo en desarrollo y su ambiente, tiene efectos a largo plazo sobre el proceso de conectividad del SNC y también consecuencias decisivas para la vida futura del hombre y de los animales.

Factores genéticos y desarrollo cerebral

Hace más de 200 años existía la creencia de que el individuo adulto era preformado en el esperma o en el huevo y que su desarrollo, consistía simplemente en el desdoblamiento de esas características en el útero materno. En cierta forma los hallazgos recientes en genética molecular han conducido a revivir nuevamente este pensamiento preformístico. Así el código genético se considera como un sistema biológico molecular que guarda la información vital para la supervivencia de la especie en su ambiente natural. Esta información se decodifica progresivamente dentro del organismo durante su desarrollo ontogenético. Aunque ciertamente se conoce que los factores genéticos influyen sobre el crecimiento y la diferenciación celulares, sin embargo, actualmente se acepta que el desarrollo postnatal, también depende en gran medida del impacto que los estímulos medioambientales no programados, ejercen sobre las neuronas durante su fase de mitosis, migración, crecimiento y diferenciación. En los últimos años, esta área de la ontogenia neural,

ha tenido un avance explosivo, de tal modo que su revisión por si sóla, ameritaría un verdadero tratado y que desde luego no es el propósito de esta revisión.

Desarrollo y sistemogénesis

Es difícil dar una definición de desarrollo que satisfaga e incluya los usos de este término en diferentes campos. Sin embargo, referido a los procesos biológicos, el término se puede considerar como la serie de cambios secuenciales que transforman cualquier sistema biológico de organización relativamente simple, en un sistema de complejidad y diferenciación progresivas, hasta que se alcanza un estado de relativa estabilidad. Esta definición también pudiera en cierta forma corresponder al término de ontogenia.

Al nacimiento la mayor parte de los mamíferos presenta una notable inmadurez motora, sensorial y homeostática (Adolph, 1957). Esta condición pone en un serio compromiso su supervivencia, ya que con una notable inmadurez los recién nacidos, tienen que enfrentarse a diversos cambios medioambientales del tipo de las carencias nutricionales, los cambios térmicos, la agresión de los depredadores, las variaciones climatológicas, la desnutrición, el dolor de diferentes orígenes, etc. El desarrollo ontogenético en cualquier especie, sigue un patrón determinado que le permite al recién nacido un máximo de supervivencia en la naturaleza. Así, aquellas partes o estructuras del sistema nervioso que son necesarias para el control de las funciones vitales en el momento del nacimiento, maduran significativamente más rápido que el resto. Este es el concepto de "sistemogénesis" introducido al campo de la Biología del Desarrollo por Anokhin (1964a), quien señala por ejemplo, que las estructuras nerviosas que gobiernan los reflejos de prensión y de succión están ya bastante desarrolladas al nacimiento, con lo cual se asegura que las necesidades de acercamiento y contacto con la madre y las de nutrición sean ampliamente satisfechas (Fig. 1). Si la visión es esencial desde el nacimiento para el patrón de vida de la especie en su habitat natural, los infantes nacerán con los ojos abiertos y funcionalmente activos. De igual manera, si la función olfatoria es de capital

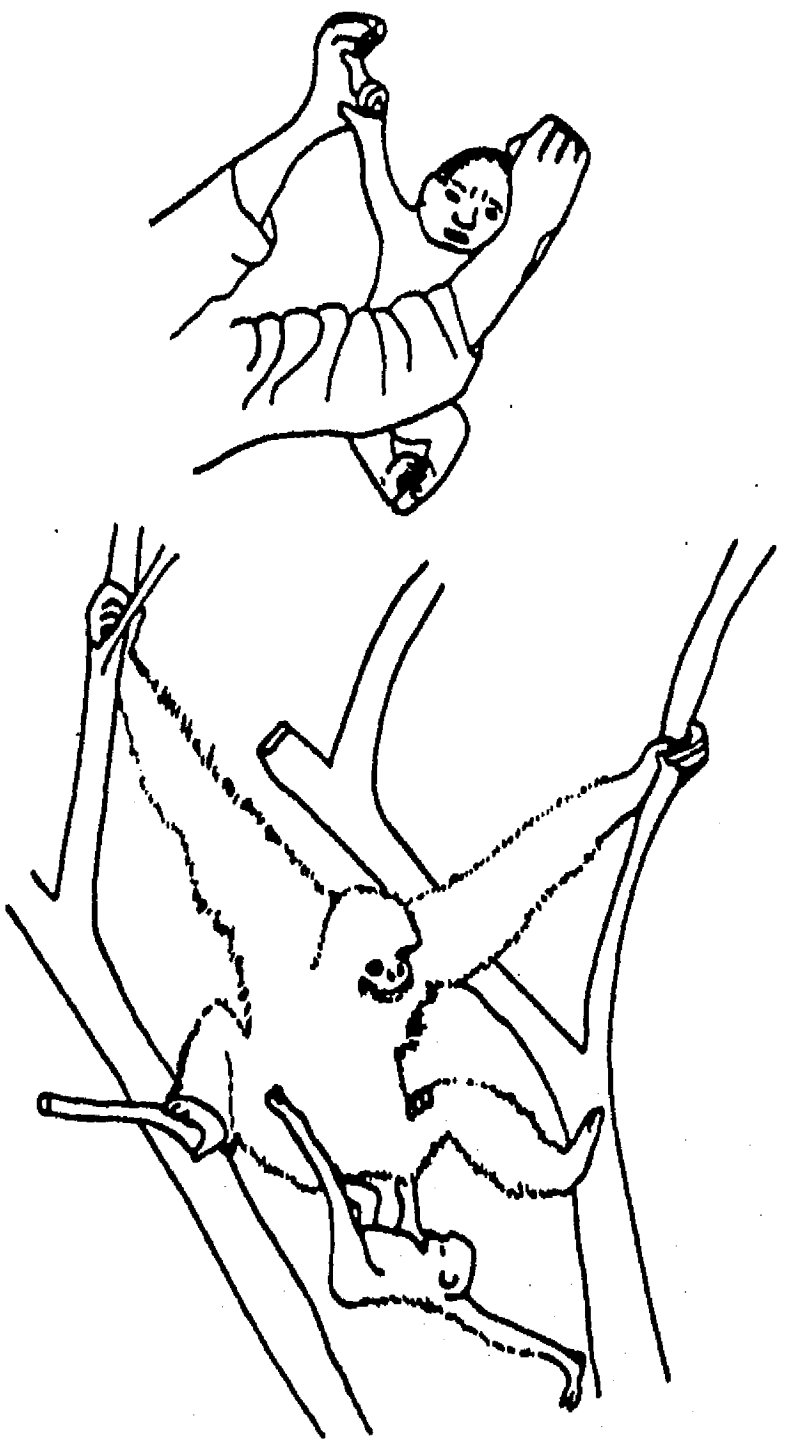


Fig. 1. Reflejo de prensión en el niño recién nacido y en el infante de gibón. En ambos casos, el cuerpo de éstos puede suspenderse en el aire mediante la prensión con los dedos de sus extremidades superiores, a puntos de apoyo en el espacio. El vigor de la respuesta refleja permite al lactante, sujetarse a la madre para favorecer su nutrición y su transporte en el medio ambiente.

importancia para el reconocimiento de la madre y del ambiente del nido, los sujetos nacerán con parte de dicha función desarrollada mientras que otros sistemas sensoriales de importancia secundaria se desarrollarán más lentamente. No obstante que durante el desarrollo postnatal algunas estructuras nerviosas avanzan a otras en su desarrollo (heterocronía), el cuidado maternal durante esta etapa juega un papel fundamental para el crecimiento ulterior del recién nacido, hasta que éste es capaz de bastarse a sí mismo.

Periodo crítico

Se conoce ampliamente que en la etapa perinatal de la mayor parte de los mamíferos, hay un periodo breve durante el cual, diferentes tipos de estímulos ambientales, provocan marcados cambios en la morfología y el funcionamiento del SNC en desarrollo, los cuales se hacen evidentes cuando el organismo es adulto. A este período de mayor vulnerabilidad del SNC se le ha llamado "periodo crítico", "periodo sensitivo" o "periodo de rápido desarrollo cerebral", dado que un mismo tipo de estimulación, antes o después de este intervalo, tiene escasos o nulos efectos sobre los centros nerviosos en desarrollo (Dobbing, 1972; Morgane, 1992).

La idea de la existencia de periodos críticos durante el desarrollo cerebral, deriva de algunos conceptos etológicos y neurobiológicos como el fenómeno de la impronta (imprinting), que se observa en las aves recién nacidas, en las cuales ocurre una fijación conductual usualmente dirigida hacia un miembro o a los miembros de una misma o de diferente especie (Lorenz, 1935). Asimismo, este concepto posiblemente sea análogo al "periodo de socialización" que ocurre en el desarrollo postnatal de algunos mamíferos como el perro (Fox y Stelzner, 1966; Scott, 1968). Por otra parte en Neurobiología, se habla del "periodo crítico" para describir un estado de desarrollo en el que diversos fenómenos físicos tales como la apertura de los párpados y los meatos auditivos externos, el inicio del EEG y la actividad eléctrica provocada, etc., aparecen bruscamente y en forma significativa (Flexner, 1955). Diversos estudios de la literatura han establecido que la causa de

esta vulnerabilidad cerebral, obedece a la concurrencia temporal de factores programados como los procesos de sinaptogénesis, migración, diferenciación, mielogénesis, gliogénesis y mielinización con los factores no programados (Morgane, 1992). Así, si los estímulos medioambientales o epigenéticos actúan cuando estos procesos citogenéticos son más intensos, entonces la posibilidad de interferencia en el desarrollo cerebral y funcional será mayor; la inversa también es cierta (Fig. 2).

Al nacimiento hay diferencias importantes en la conducta de los animales que reflejan su distinto grado de maduración. En general la mayoría de los mamíferos se pueden clasificar en dos grandes grupos: a) los que nacen relativamente maduros (precociales) y son capaces de defenderse y enfrentarse al ambiente por sí solos; y b) los que nacen inmaduros (altriciales) y necesitan de un gran cuidado materno para sobrevivir. Así por ejemplo es bien conocido que al nacimiento, los infantes del caballo, el cuyo, la cabra, la vaca y otras especies son capaces de moverse libremente, sus ojos y oídos están abiertos y funcionalmente activos. En estas especies bajo circunstancias favorables y con un cuidado materno relativamente escaso, los infantes se bastan por sí solos para sobrevivir. En cambio, la rata, el ratón, el conejo, el perro, el gato y otras especies, incluyendo el hombre, al nacer, tienen una marcada inmadurez homeostática, motora y sensorial y requieren de un cuidado materno muy estrecho para alcanzar su completo desarrollo (Adolph, 1957). El hombre está incluido dentro de este último grupo y por esta razón, la información experimental derivada de estas especies, suele ser de gran utilidad para la mejor comprensión de las causas que afectan el desarrollo cerebral del ser humano.

Conducta y adaptación

Desde el punto de vista funcional la conducta es la expresión, en mayor o menor grado, de la actividad de las estructuras biológicas que constituyen al organismo. La mayoría de los patrones de conducta animal tienen como finalidad la supervivencia del individuo y de la especie en la naturaleza. Ejemplos claros de este objetivo son la huída de una presa ante su depredador, la función reproductora cuya

Gliogénesis

Mielinización

Macroneurogénesis

Microneurogénesis

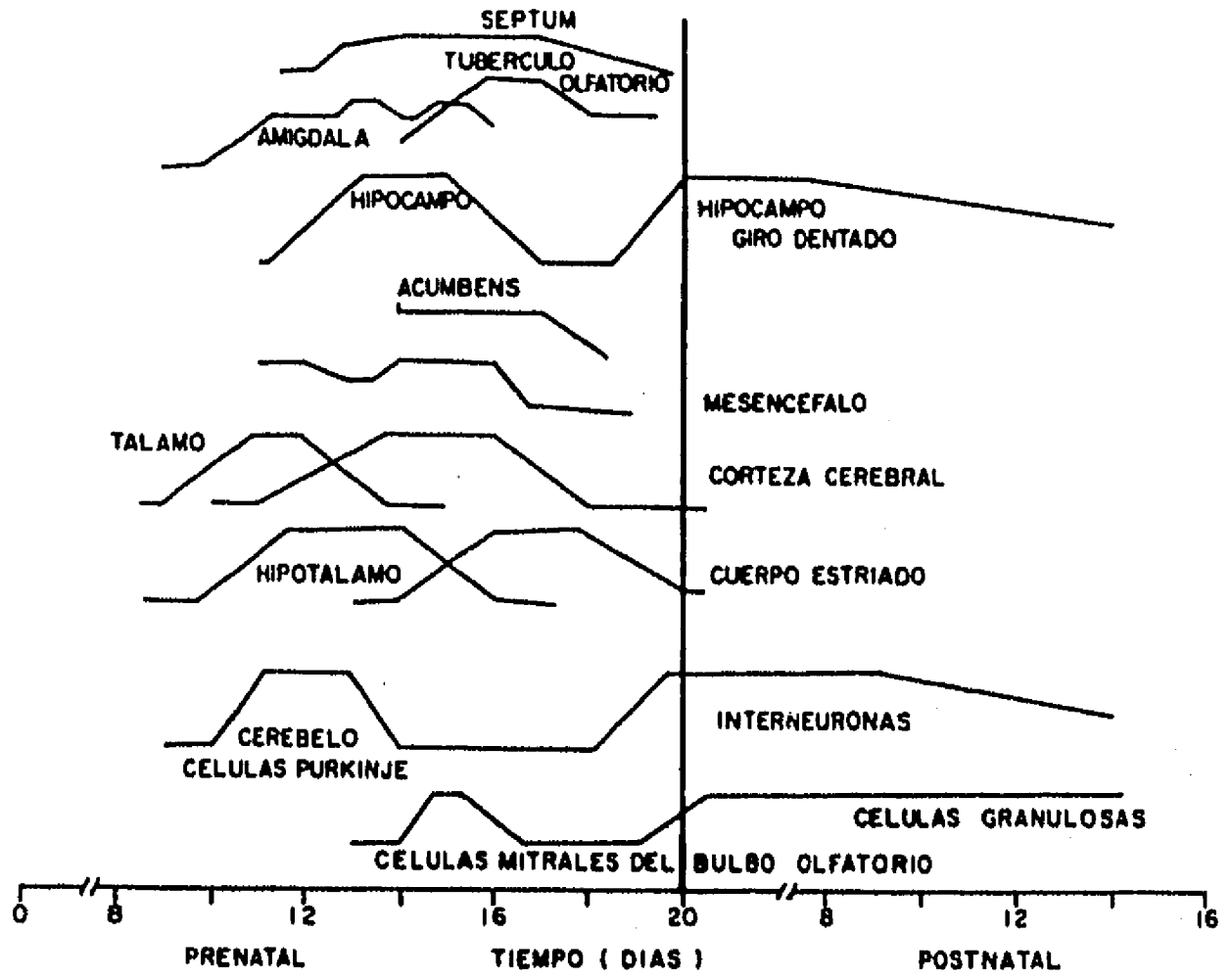


Fig. 2. Macroneurogénesis y microneurogénesis en distintas estructuras del SNC durante el período prenatal y postnatal en la rata. La macroneurogénesis ocurre durante la etapa prenatal, mientras que la microneurogénesis y la gliogénesis, son eventos que se inician al final del periodo prenatal y culminan durante el postnatal; la mielinización se ubica durante la etapa postnatal. El esquema muestra el carácter heterocrónico de las estructuras neurales durante el proceso del ensamblaje cerebral en la ontogenia. Modificado de P. M. Rodier y S. S. Reynolds, Exper. Neurol., 57: 81-93, 1977.

capacidad generalmente es muy grande en especies dotadas con frágiles mecanismos de defensa o con deficientes mecanismos adaptativos hacia el ambiente, etc. La supervivencia de cualquier especie en su ambiente natural implica el desarrollo, a través de la evolución, de variados mecanismos de adaptación que permiten su existencia en el ambiente. Esta adaptación es continua y es la que determina el ajuste del organismo como un todo, no sólo a los estímulos del ambiente exterior, sino también a los requerimientos de su medio interno.

Hay dos mecanismos por los que la adaptación se lleva a cabo: a) aquel en el que la especie, a través de los procesos de mutación y selección natural, logra la adaptación asegurando así su supervivencia. La información ambiental obtenida por estos procesos, se transmite de padres a hijos entre los miembros de una misma especie por medio de los genes en los cuales está codificada. b) El segundo mecanismo consiste en la acción recíproca instantánea entre el individuo y los estímulos provenientes de sus alrededores. Por este mecanismo, se logra la adaptación instantánea de la conducta del individuo a los requerimientos momentáneos de su medio circundante.

En los últimos años se ha puesto de manifiesto que la interacción que determina la adaptación instantánea en el adulto, durante el periodo postnatal inmediato juega, un papel fundamental en los procesos de organización morfológica y funcional del SNC del infante. La versatilidad y la eficiencia de funciones complejas del SNC tipo aprendizaje, memoria, reactividad emocional, estados de conciencia, etc., que caracterizan a un organismo adulto, dependen posiblemente de los límites de crecimiento y complejidad del SNC, establecidos por la acción de diversos factores ambientales sobre las neuronas en desarrollo, en conjunción con la información genéticamente transmitida para cada especie.

Efectos de las hormonas neurotrópicas

Durante la ontogenia, el desarrollo del tejido cerebral es altamente dependiente de la acción de varias hormonas denominadas neurotrópicas, que incluyen a las hormonas tiroideas, la hormona de crecimiento, las hormonas gonadotrópicas y los corticoides suprarrenales, que por diferentes mecanismos son capaces de interferir con la organización citoarquitectónica y la función cerebral. Enseguida se mencionan las acciones mejor conocidas de las alteraciones en la concentración de las hormonas mencionadas durante el periodo perinatal.

Hormonas tiroideas y desarrollo neural

El nivel de las hormonas tiroideas circulantes en el medio interno, es una de las influencias del ambiente que más se ha estudiado y que se sabe afecta seriamente el desarrollo cerebral. Así, de los estudios clásicos de Eayrs y sus asociados (1955, 1960, 1961, 1964; Eayrs y Taylor, 1951; Eayrs y Horn, 1955), se conoce que una deficiente secreción tiroidea durante el periodo de rápido desarrollo cerebral en la rata, provoca un marcado retraso en el desarrollo morfológico y electrofisiológico del SNC y por lo tanto de la conducta. En el hipotiroidismo las neuronas de las diversas áreas corticales son comúnmente pequeñas, con escasas y delgadas ramificaciones dendríticas, un reducido número de espinas dendríticas (Fig. 3) y neuronas que muestran un claro retardo en el depósito de la mielina axonal (Eayrs, 1960; Hamburgh, 1966, 1968; Hamburgh y col., 1977; Ruiz-Marcos y col., 1979, 1982). La amplitud y la frecuencia del electroencéfalograma están reducidas, la actividad eléctrica cortical provocada por la aplicación de estímulos sensoriales se caracteriza por la presencia de potenciales provocados pequeños de gran latencia y fácil fatigabilidad a la estimulación iterativa (Bradley y col., 1960). La excitabilidad cerebral se encuentra alterada, presentando ciertos grupos neuronales una reducción en el umbral para provocar crisis convulsivas por estimulación eléctrica. Estas alteraciones en la excitabilidad, van asociadas a cambios en la actividad de la bomba sodio/potasio en el tejido cerebral, que interfieren con los procesos de

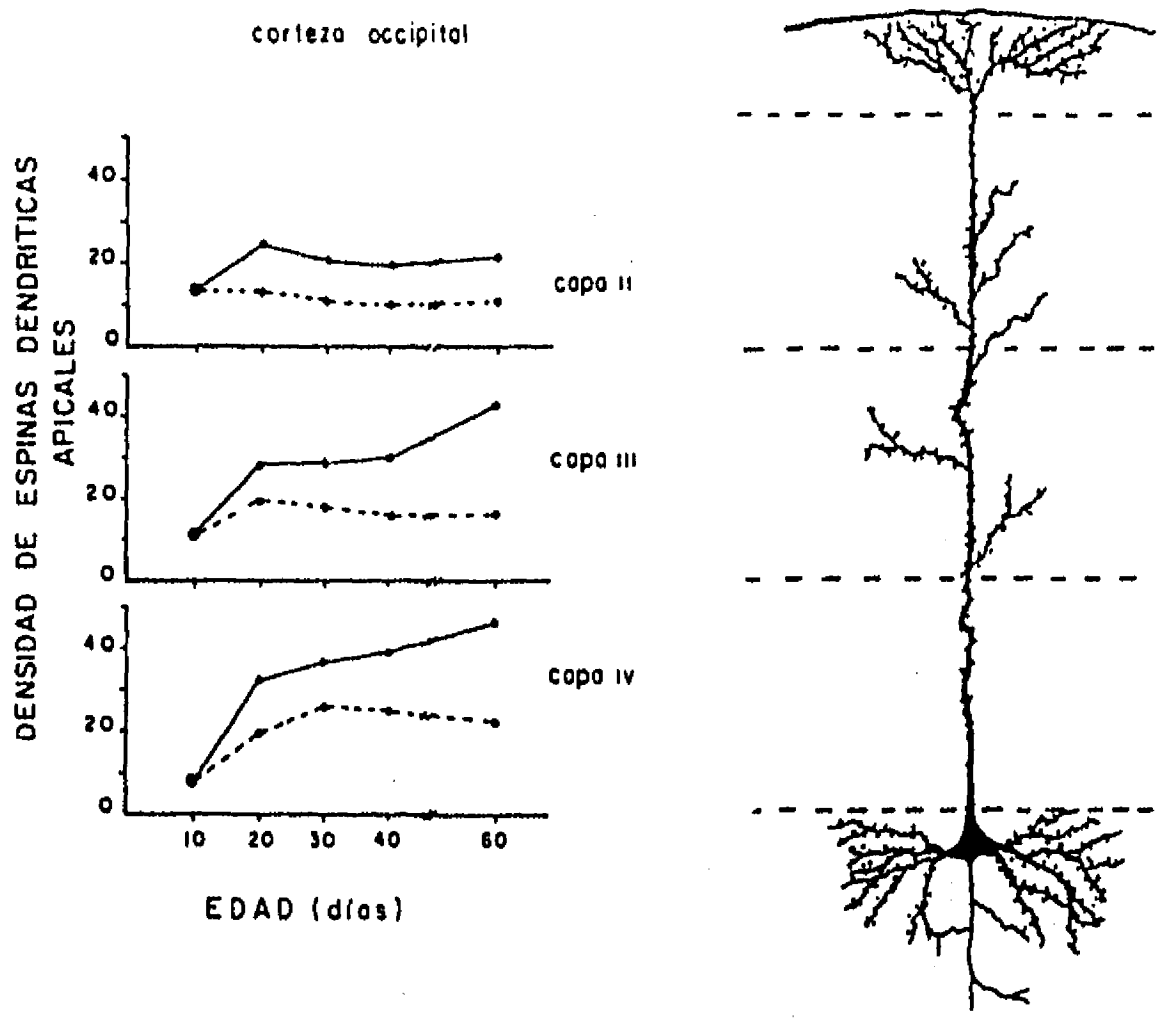


Fig. 3. Desarrollo de la densidad de espinas dendríticas en la prolongación apical (segmentos de 50 μ) de neuronas piramidales grandes de la V capa cortical (derecha), a lo largo de su trayecto por las capas II, III y IV de la corteza visual primaria de la rata. Las neuronas de las ratas hipertiroideas (línea punteada en las gráficas), tienen un menor número de espinas a lo largo del desarrollo con respecto a sus testigos (líneas continuas). Modificado de M. Salas y col., Ontogenia Neural. 177-198, 1991.

polarización y repolarización de las membranas neuronales (Valcana y Timiras, 1969). La reacción de orientación y sobresalto así como otros patrones conductuales también se retrasan notablemente en el desarrollo (Bradley y col., 1960). Sin embargo, una vez que ha transcurrido el periodo de rápido desarrollo cerebral, la tiroidectomía y la deficiencia tiroidea no producen ningún efecto significativo o bien las alteraciones morfológicas y funcionales son mínimas (Eayrs, 1964).

Por el contrario un exceso de tiroxina (1µg/gr de peso corporal) administrado intraperitonealmente durante los 4 a 5 primeros días postnatales de la rata, acelera la aparición y la maduración de algunos de los índices empleados para valorar el proceso de desarrollo (Schapiro, 1968; Salas y Schapiro, 1970; Schapiro y col., 1973; Davenport y Gonzalez, 1973; Salas y col., 1976, 1977, 1993), tales como el número de espinas dendríticas de las células piramidales corticales (Schapiro y col., 1973), el proceso de mielinización y la concentración de colesterol y de acetilcolinesterasa cerebrales (Hamburgh, 1966; Schapiro, 1968; Hamburgh y col., 1977). Asimismo, existe un desarrollo precoz de la actividad electrocortical espontánea y también de la provocada por la aplicación de estímulos sensoriales (Schapiro y Norman, 1967; Salas y col., 1977), así como de la actividad de nado (Schapiro y col., 1970), la reacción de orientación, de sobresalto y el aprendizaje (Eayrs, 1964; Schapiro, 1968). En estudios posteriores, se ha señalado que en la rata, la maduración precoz del SNC producida por un exceso de tiroxina, solamente se presenta hasta los 20 días del nacimiento y que a partir de esta edad, se observan efectos completamente opuestos debido posiblemente a la reabsorción de espinas, dendritas y de neuronas en diversas áreas corticales y subcorticales (Eayrs, 1964; Schapiro, 1968; Salas y col., 1977, 1993). Este doble efecto provocado por la tiroxina aplicada en etapas tempranas del desarrollo en los mamíferos, parecería ser una reminiscencia de los efectos observados durante el fenómeno de la metamorfosis de los anfibios (Hamburgh, 1968; Grave, 1977). A pesar de todo este conjunto de evidencias experimentales, el efecto dual de las hormonas tiroideas sobre el SNC en crecimiento y sus mecanismos de acción, aún son motivo de intenso estudio.

Efectos de las hormonas gonadales

En el sistema gonadal se ha señalado que un exceso de esteroides durante el periodo neonatal, altera el patrón de desarrollo de la conducta sexual y probablemente también la cantidad de hormonas secretadas en el estado adulto. Se ha sugerido que en el infante, los esteroides tienen un papel organizador de feminización o de masculinización sobre los centros nerviosos hipotalámicos que controlan la conducta sexual, mientras que en el adulto tienen solo un carácter excitador sobre estas mismas estructuras (Harris, 1964; Young y Goy, 1964). Por ejemplo, la administración de estrógenos o de progesterona en ratas adultas macho, castradas inmediatamente después del nacimiento, provoca la conducta sexual típica femenina. Esto no sucede en el caso de animales que son castrados después de los 5 primeros días del nacimiento. La administración de grandes dosis de testosterona o de estrógenos a ratas hembra durante las primeras 12 horas después del parto, impide la respuesta normal a las hormonas sexuales femeninas durante la vida adulta (Harris y Levine, 1965). Paradójicamente las hembras tratadas con estrógenos en la infancia, pueden mostrar tanto patrones de conducta sexual masculina como respuesta a la administración de testosterona durante la vida adulta. En cambio los animales no tratados con estrógenos solamente muestran conducta de monta cuando se les inyecta la misma cantidad de testosterona (Levine y Mullins, 1968). Asociado a estos efectos de las hormonas gonadales sobre la conducta sexual, se ha descrito que la administración de dosis altas de estrógenos pudieran acelerar el desarrollo del SNC. Así por ejemplo tomando a las respuestas transcallosas y a los potenciales de la pirámide bulbar como criterios para valorar la maduración del SNC, se ha encontrado que las ratas tratadas con estradiol en los primeros días del nacimiento, presentan respuestas de latencia corta y de gran amplitud en comparación con las ratas testigo. Estos hallazgos sugieren que el estradiol pudiera acelerar los procesos de mielinización o de las conexiones sinápticas aumentando así la excitabilidad y la conducción de impulsos nerviosos en el cerebro (Valcana y Timiras, 1969; Curry y Timiras, 1972; Arai y col., 1986).

Hormona del crecimiento (GH) y desarrollo cerebral

La GH es uno de los reguladores primarios del crecimiento cerebral, que a dosis farmacológicas durante la preñez de la rata, estimula el crecimiento del cerebro de las crías. En este sentido se ha encontrado que ocurre un incremento del peso y del contenido del DNA cerebral. También se ha descrito que la somatotrofina puede afectar el peso corporal solamente en las madres y progenie de bajo peso . Por esta razón para estudiar el efecto de la hormona GH sobre el desarrollo cerebral, se ha empleado el modelo de la restricción nutricional de ratas embarazadas. Así, conociendo la participación de la GH en el metabolismo de los carbohidratos y la importancia de la glucosa como fuente de energía para el feto, Zamenhof y asociados (1971), evaluaron el efecto de la restricción calórica en ratas embarazadas y el efecto de la administración de GH sobre el desarrollo cerebral prenatal. Los hallazgos mostraron que la restricción calórica en ratas gestantes durante los días 10 al 20 de la gestación, resulta en una reducción significativa en el peso corporal, en el peso de la placenta, en el peso cerebral y en el contenido del DNA y proteínas cerebrales de las crías recién nacidas. Estas reducciones no se presentaron si las madres con dieta restringida, se trataban concomitantemente con GH de bovino. Los autores postularon que debido a que la GH no cruza la barrera placentaria, entonces un posible efecto de la hormona sería a través de la movilización de reservas nutricias de la madre hacia sus crías. Sin embargo, la progenie de ratas mantenidas bajo una dieta normal no mostraron ningún aumento significativo en el contenido de DNA cerebral cuando se trataron en forma semejante.

En otros estudios (Sara y col., 1974), se han analizado los efectos de la GH sobre el crecimiento fetal y placentario cuando la hormona se administraba bajo condiciones de control nutricional, para determinar el mecanismo de acción de la hormona. Los resultados indicaron que la GH, incrementó el peso cerebral y el contenido de neuronas cerebrales, sin cambios en el peso corporal fetal, lo que sugirió una acción selectiva de la hormona sobre el crecimiento cerebral. Por lo

tanto, este efecto no pudo ser atribuido a la movilización de los nutrimentos provenientes de la madre. Sin embargo, en la madre se pudo reconocer un crecimiento de la placenta, lo cual apoyó la idea de una regulación placentaria del crecimiento fetal. Con fundamento en estos hallazgos puede existir la alternativa de que un mensajero secundario como la somatomedina o una substancia trófica similar, posiblemente de origen placentario, sea capaz de mediar el crecimiento cerebral del feto.

En otros experimentos se ha mostrado un incremento en el peso cerebral y en el número de neuronas corticales, determinado por la incorporación de timidina marcada al DNA, seguida de autorradiografía. En el estado adulto de estos mismos animales, se encontró que el desempeño en el aprendizaje de una serie de tareas de discriminación, aumentó significativamente con respecto a los animales testigo cuyas madres no fueron tratadas con la GH durante la gestación (Sara y Lazarus, 1975).

El retardo en el crecimiento corporal del ratón enano hipofisiario (dw, Snell) es producido generalmente por la deficiencia de GH y de otras hormonas similares. En este modelo se ha encontrado una deficiente mielinización asociada al enanismo característico, la cual es producida por una reducción en la proliferación de oligodendrocitos (Noguchi y col., 1982). Estos efectos se han asociado a una reducción significativa en la velocidad de conducción de los impulsos nerviosos a lo largo de los nervios, que interfiere con la descarga neuronal aferente hacia las estructuras nerviosas supraespinales (Fuhrman y col., 1986).

Corticoides suprarrenales y desarrollo cerebral

Varios estudios han señalado que la administración de una dosis de 1 mg de acetato de cortisol por vía subcutánea en la rata recién nacida, provoca como alteraciones principales las siguientes: retardo en el depósito de la mielina, reducción en el número de las espinas dendríticas de las neuronas piramidales corticales y decremento en la concentración de colesterol y acetilcolinesterasa cerebrales

(Schapiro, 1968). Asimismo, origina un retardo en la maduración de la actividad eléctrica espontánea y de la provocada por la aplicación de estímulos sensoriales (Salas y Schapiro, 1970). Todos estos cambios están asociados a un bajo rendimiento en pruebas de condicionamiento y también en una alterada reacción al estrés (Schapiro, 1968; Salas y Schapiro, 1970; Peruzovic y Milkovic, 1986). Con respecto a la conducta refleja se sabe que se produce un retardo en el desarrollo de la conducta de nado (Schapiro y col., 1970) y que la reacción de sobresalto se encuentra incrementada.

Factores de Crecimiento

Los postulados de la doctrina de la neurona establecidos por Cajal, indican que las neuronas constituyen la unidad trófica que mantiene la integridad de los circuitos en los que se ubican. Sin embargo de la información generada en los últimos 25 años, se sabe que durante el desarrollo ontogenético, los impulsos nerviosos que se transmiten a lo largo de las neuronas en crecimiento, liberan en sus axones terminales factores tróficos que promueven el crecimiento neuronal. Estos factores son péptidos de bajo peso molecular producidos endógenamente, que estimulan el desarrollo neuronal. Dentro de este grupo de factores destaca el factor nervioso de crecimiento (NGF), identificado inicialmente por Bueker en 1948 y después caracterizado por Levi-Montalcini y Hamburger, 1951, 1953. De estos estudios se pudo establecer que tanto las células de los ganglios sensoriales como las del sistema simpático, crecían exageradamente cuando se colocaban en la vecindad de células tumorales de sarcoma. Con posterioridad se identificó que el NGF, también era secretado en el veneno de serpientes, así como en las glándulas submaxilares del ratón y otras especies (revisado por Purves y Lichtman, 1985).

La purificación y caracterización del NGF, la identificación del gen que lo genera, así como los receptores de baja y de alta afinidad a los cuales se une este factor, han hecho avanzar enormemente nuestro conocimiento sobre sus acciones promotoras del crecimiento neural (Guroff, 1993). Por otra parte, su localización en

cantidades minúsculas en casi todos los tejidos del organismo y en cantidades mayores en las neuronas ganglionares del simpático y sensoriales y sus órganos blanco, indican que este factor es esencial para la maduración y la supervivencia de las neuronas de la médula espinal, de las células de la médula suprarrenal y de otras neuronas de diversas regiones del SNC (ver Purves y Lichtman, 1985; Guroff, 1993).

El hecho de que en aquellas regiones del SNC donde el NGF no actúa, sea posible el mantener las neuritas y la reparación en la conectividad neuronal que sigue a la degeneración, indica que es posible que existan otros factores similares al NGF que desempeñen funciones tróficas, tal podría ser el caso de los factores de crecimiento que actúan en el sistema parasimpático (Barde y col., 1983).

Otra familia de péptidos involucrados en el desarrollo neuronal, es la de los factores de crecimiento de tipo insulínico (IGF), los cuales se encuentran constituidos por la insulina, el IGF-I y el IGF-II. Comúnmente a la insulina se la relaciona con el metabolismo de los carbohidratos, mientras que a los IGFs se les asocia con los procesos de crecimiento y diferenciación celulares. La mayor parte de los IGFs se sintetizan principalmente en el hígado y actúan por mecanismos parecidos a las hormonas. Aunque otros tejidos también los producen, éstos a diferencia de los anteriores, actuán a nivel local y de manera autócrina o parácrina. Los receptores a la insulina y al IGF-I estructuralmente son muy similares, a pesar de estar producidos por distintos genes. Están formados por 2 subunidades (α y ß) unidos por puentes disulfuro. Las subunidades α son extracelulares y son las responsables de la unión con los factores, mientras que las ß son transmembranales y contienen un dominio a tirosina cinasa en sus porciones intracelulares. La unión del ligando a la subunidad α, activa a la tirosina cinasa de la subunidad ß. Los receptores a IGF-II difieren de los insulínicos y los IGF-I, en que constan de un dominio largo extracelular y de un dominio corto intracelular que no tiene la actividad tirosina cinasa. Paralelamente, los receptores IGFs se expresan en estadios tempranos de la embriogénesis, lo cual indica que participan en los procesos de diferenciación neural. Los receptores IGF-I son los más abundantes durante el desarrollo neural, lo cual

sugiere que son esenciales para la supervivencia neuronal. Paralelamente se ha establecido que el IGF-I, estimula la síntesis de DNA y RNA en las neuronas de cultivo de tejidos, apoyando lo anterior la supervivencia de las neuronas y el crecimiento de las neuritas (Recio-Pinto e Ishi, 1988; Bondy y Wei-Hua, 1993).

Efectos de la estimulación sensorial en la ontogenia neural

En los pasados 50 años, se ha hecho evidente que algunos estímulos del medio ambiente tales como el sonido, la luz, la estimulación vestibular, la propioceptiva, la somestésica, la olfatoria, etc., desempeñan un papel importante en el proceso de maduración del substrato neural. Hebb en 1949, desarrolló el concepto de que el mayor número de interconexiones neuronales, podía ser la base estructural de una mayor capacidad funcional del cerebro. De sus estudios surgió la hipótesis de que un exceso de información, provocado por un exceso en el manejo de los animales durante el periodo neonatal, podría acelerar la maduración tanto de los relevos sinápticos como de las áreas de integración sensorial del SNC. En otras investigaciones se ha sugerido que esta aceleración en el crecimiento neuronal, podría ser el resultado de un incremento en el metabolismo neuronal provocado por el exceso de impulsos nerviosos aferentes. Este incremento metabólico produciría un aumento en el flujo sanguíneo cerebral, que al aumentar el aporte de nutrimentos al tejido neuronal inmaduro, permitiría un mejor crecimiento y capacidad para el establecimiento de las interconexiones neuronales (Schapiro y Vukovich, 1970). Apoyan esta hipótesis, diversos estudios conductuales en los cuales se ha reportado que los animales adultos que fueron estimulados por medio de manoseo durante la infancia temprana, son emocionalmente más estables (orinan y defecan menos), exploran más libremente cuando son colocados en un ambiente novedoso y aprenden mejor ante diversas pruebas de condicionamiento (Denenberg, 1964). Asimismo, la respuesta adrenocortical también se reduce como resultado de la manipulación sensorial previa (Walker y Aubert, 1988).

En un estudio realizado en niños lactantes africanos, que por razones económicas y sociales, viajan por periodos prolongados sobre las espaldas de sus padres y reciben una gran estimulación vestibular y propioceptiva, al año de vida ya son capaces de caminar, correr y realizar diversas maniobras de equilibrio sobre una superficie pequeña, que requiere de una avanzada coordinación muscular y habilidad motora (Newton y Levine, 1968). Esto contrasta con el comportamiento de los niños europeos y de los africanos extraídos del mismo núcleo social pero crecidos en Europa, en los cuales esas mismas maniobras requieren de mayor tiempo para realizarse. Por otra parte, diversos estudios morfológicos muestran que los animales manipulados antes del destete, presentan un mayor grosor y peso de la corteza cerebral, así como un aumento en el número de las dendritas y de los procesos espinosos de las neuronas piramidales grandes de la neocorteza (Diamond y col., 1964; Rosenzweig y col., 1969; Ruíz-Marcos y Valverde, 1969; Schapiro y Vukovich, 1970; Green y col., 1983; Pascual y col., 1993). Esta aceleración en el crecimiento neuronal, va asociada a un incremento en el desarrollo de los potenciales provocados por la aplicación de estímulos sensoriales (Leah y col., 1985). En experimentos de privación sensorial en la rata, cuando se les coloca en espacios físicos reducidos con ausencia de luz y sonido por varios días, se han encontrado cambios morfológicos opuestos, particularmente en el área visual (Valverde, 1967; Rosenzweig y col., 1969). A pesar de lo consistente de estos resultados se requiere de una mayor información morfológica, electrofisiológica y bioquímica que complemente y de bases más firmes a esta hipótesis.

Privación perinatal de nutrimentos y ontogenia neural

La desnutrición como resultado de la deficiente ingestión en la cantidad y calidad del alimento, es el factor del ambiente externo que más frecuentemente afecta el desarrollo cerebral y que a su vez, es causa primordial de los altos índices de mortalidad infantil en el mundo. Según la opinión más generalizada, cuando se habla de desnutrición se está considerando aquella condición en la que existe una notable reducción en la ingestión de proteínas fundamentalmente (McCance y

Widdowson, 1968). Aunque si bien es cierto que la desnutrición como cuadro clínico, usualmente implica el aporte reducido de varios elementos de la dieta que le dan el calificativo de síndrome pluricarencial. Parecería ser entonces que la privación parcial de alimento, podría ser la condición más común que se da en los países pobres y en vías de desarrollo, y no la desnutrición de un sólo elemento de la dieta que parecería ser quizás el tipo más frecuente en los países desarrollados en los que la demanda por el alimento "chatarra" o de elaboración rápida, prevalece sobre la ingesta de una alimentación balanceada.

Por estudios llevados a cabo en niños, se sabe que el cerebro presenta una gran vulnerabilidad a los efectos de la desnutrición y que el crecimiento cerebral no sólo se retrasa sino que también puede detenerse (Mourek y col., 1967; Von Muralt, 1972). De las lamentables experiencias obtenidas durante la segunda guerra mundial, se sabe que los individuos que murieron por la falta de ingestión de alimento en los campos de concentración, presentaban una pérdida del 3% del tamaño del cerebro y del corazón. Asimismo, los músculos esqueléticos, el hígado y el bazo se encontraban reducidos en un 31%, 54% y 67% respectivamente (Von Muralt, 1972). De estos estudios se ha concluido que los órganos que son esenciales para la supervivencia, son poco afectados en comparación con los restantes, es decir, que los tejidos son sacrificados en proporción inversa a su importancia funcional para el mantenimiento de la homeostasia. Esta circunstancia pudiera explicarse debido a que existen mecanismos homeostáticos que protejen al cerebro y al corazón durante la privación aguda o crónica de alimento, justamente debido a su alta vulnerabilidad. Otra posibilidad es que esta secuencia de efectos pudiera ser mayor en el hígado y el bazo y menor en el corazón y el cerebro. Esta condición cierta en el adulto, opera aparentemente dentro de límites más reducidos en el infante, cuyos mecanismos homeostáticos están aún en fase de desarrollo y tal situación determina una mayor posibilidad de daño cerebral.

HALLAZGOS MORFOLOGICOS

Mediante experimentos realizados en ratas, ratones y cerdos, se conoce que la restricción en la ingesta de alimento desde el nacimiento hasta el momento del destete, produce en general una reducción permanente en el peso cerebral y en el contenido de agua corporal (Culley y Lineberger, 1968). Asimismo, diversos experimentos han sugerido que la privación neonatal de alimento reduce importantemente el depósito de la mielina en el SNC (Davison y Dobbing, 1966; Bass y col., 1970). Estudios de autorradiografía han mostrado que la desnutrición impide también la diferenciación y la migración de los oligodendrocitos que están involucrados en el proceso de la mielinización. En casos de desnutrición perinatal las neuronas permanecen acumuladas en las cercanías de las paredes ependimarias de los ventrículos cerebrales, o bien se encuentran diseminadas en el trayecto de sus rutas de migración, y en las áreas en las que ellas normalmente se distribuyen (Bass y col., 1970).

Impactos sobre la neocorteza

Actualmente, son bien conocidos los cambios que ocurren normalmente tanto en las células como en la red de fibras nerviosas de las áreas sensoriales de la corteza cerebral durante el desarrollo de la rata (Bass y col., 1970; Jacobson, 1970; Salas y col., 1974). Al nacimiento, esta área del telencéfalo de la rata tiene un grosor promedio de 900 micras, contiene núcleos de neuronas indiferenciadas y aún no se inicia el proceso de la mielinización. A los 10 días de edad, la corteza de las ratas normalmente nutridas tiene un espesor promedio de 1650 micras, las 6 capas corticales clásicamente conocidas están bien definidas y la densidad neuronal está reducida en comparación con la que se observa al nacimiento. Las ratas desnutridas durante un periodo de 21 días a partir del nacimiento, presentan al día 10 de edad un decremento del espesor cortical que sólo alcanza 1220 micras como promedio. Las células por unidad de volumen están estrechamente agrupadas y la estratificación de las capas aún es escasa como ocurre normalmente en un animal

de menor edad (Bass y col., 1970). Entre los 10 y los 30 días postnatales, la corteza de estos mismos animales continúa aumentada en densidad por la escasa migración celular y el patrón de conectividad aún se encuentra desorganizado a pesar de que el espesor cortical se aproxima al valor normal. El espesor alcanza los valores del animal normal entre los 10, 40 y 50 días de edad cuando el peso corporal se recupera (Bass y col., 1970). Sin embargo, el citoplasma aún es hipercromático en muchas neuronas y las dendritas apicales y el cuerpo celular están poco desarrollados (Cragg, 1972). Las fibras aferentes a las sinapsis axodendríticas corticales están pobremente impregnadas de mielina y las células gliales también notablemente reducidas (Wiggins y col., 1982; Fuller y Wiggins, 1984). La marcada celularidad de la zona germinal cercana a las paredes ventriculares, normalmente desaparece alrededor de los 20 días postnatales, pero en las ratas desnutridas aún es visible a los 50 días de edad (Bass y col., 1970).

El estudio del efecto de la desnutrición sobre el tejido nervioso en crecimiento se ha hecho también en componentes celulares más finos y así en algunos estudios (Cragg, 1972), se ha descrito que en las ratas desnutridas desde el nacimiento hasta el destete (24 ó 25 días de edad), presentan un 22% de aumento en la densidad neuronal, juzgada ésta por el número de nucléolos de las células correspondientes a la corteza visual. Asimismo, hay un número menor de ramificaciones neuronales circundando cada neurona y además, los axones terminales están reducidos en un 38% como promedio (Horn, 1955; Cragg, 1972). En otras estructuras del SNC como el cerebelo, el hipocampo y el tallo cerebral se han encontrado resultados muy similares a los mencionados (Chase y col., 1969; Mc Connell y Berry, 1978; Díaz-Cintra y col., 1981, 1984, 1991). Dado que los axones terminales forman parte de las sinapsis, el número de circuitos neuronales que pueden formarse en la corteza, está notoriamente reducido y por consiguiente, los procesos dependientes del funcionamiento de éstos pueden alterarse permanentemente.

Efectos sobre las espinas dendríticas

Tanto los estudios con microscopía de luz como los de microscopía electrónica, han revelado que las espinas dendríticas constituyen una característica morfológica distintiva de la membrana postsináptica en la corteza cerebral, cerebelo, tallo cerebral y médula espinal (Gray, 1959; Globus y Scheibel, 1967 a, b). Estas extensiones laterales de las dendritas fueron inicialmente descritas por Cajal (1899 a, b, 1900) y aunque él mismo las consideró como artefactos de fijación o de tinción, hoy día son consideradas como estructuras postsinápticas específicas en las dendritas, que incrementan las superficies de contacto sináptico y la efectividad en la transmisión interneuronal (Globus y Scheibel, 1967 a,b; Peters y Keiserman-Abramof, 1970). En el gato recién nacido, las dendritas corticales no tienen espinas y los estudios cuantitativos disponibles, indican que ellas surgen lentamente durante el desarrollo en estrecha asociación con la aparición de los patrones de la conducta, de la actividad eléctrica cerebral y de los procesos bioquímicos (Globus y Scheibel, 1967b). En la rata, las espinas también están ausentes al nacimiento y adquieren los valores del animal adulto, 20 ó 25 días después (Globus y Scheibel, 1967a). La identidad de estas estructuras como unidades funcionales de las sinapsis y su aparición gradual durante el desarrollo, sugiere que su ontogenia puede estar relacionada en alguna forma, con los eventos fisiológicos que ocurren durante la vida adulta.

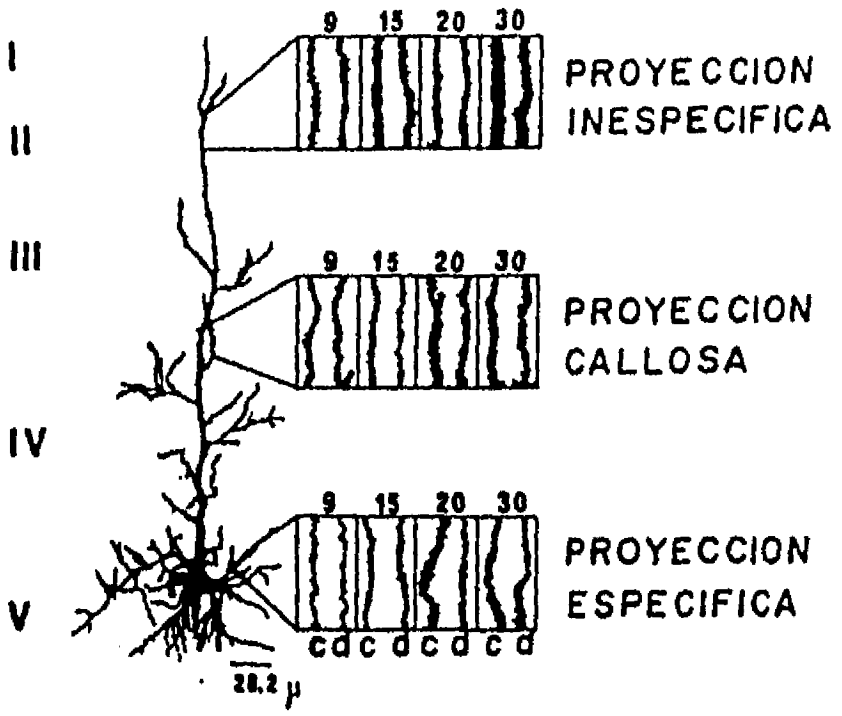
Dado que otros autores han descrito que bajo los efectos de diversas influencias ambientales del tipo de la privación o del exceso de estimulación sensorial, ocurren cambios en el número de espinas, pensamos que la privación perinatal de alimento podría igualmente alterar el número de las espinas en las dendritas de las células piramidales grandes de la corteza cerebral. Con esta idea en mente, hicimos cortes del área somatosensorial correspondiente en ratas privadas de alimento durante 12 a 14 horas diarias, durante el periodo comprendido entre el cuarto y el veinteavo días del nacimiento. Los resultados de esta investigación mostraron que tanto en los animales testigo como en los desnutridos, las espinas

dendríticas de las neuronas piramidales de la V capa de la corteza cerebral, aumenta de número progresivamente hasta el día 15 en que su valor se aproxima al de los animales adultos. Asimismo, en los animales desnutridos se apreció una reducción estadísticamente significativa en el número total de espinas, así como del grosor de los diversos tipos de dendritas y de la densidad del campo dendrítico correspondiente a las porciones basales (Salas y col., 1974; Salas y col, 1977; Salas, 1980) (Fig. 4).

Dado que las neuronas piramidales grandes representan uno de los sitios más importantes de entrada de los impulsos aferentes transmitidos a través de los sistemas tálamocorticales específico e inespecífico y del sistema calloso (Lorente de Nó, 1941), se sugirió que la desnutrición neonatal, reduce el establecimiento de nuevas interconexiones y la interacción neuronal que constituyen la base de los procesos fisiológicos complejos del tipo de la memoria, el aprendizaje, la conducta emocional, etc.

En años recientes se han estudiado los cambios histológicos que ocurren en la médula espinal de cerdos y perros desnutridos (Dickerson y col., 1967; Stewart y Platt, 1968). De este modo en cerdos mantenidos con dietas hipoproteínicas por espacio de 2 -3 semanas, se han creado fenómenos claros de cromatólisis en las motoneuronas grandes del cuerno anterior de la médula espinal, así como también un incremento en el número de oligodendrocitos perineurales. Dado que estas alteraciones no se manifestaron o fueron menos pronunciadas en animales rehabilitados nutricionalmente, se ha pensado que estos cambios son reversibles.

Los extensos estudios bioquímicos y morfológicos llevados a cabo por Winick y sus asociados (1977), han mostrado que la desnutrición detiene los procesos de división celular en el cerebro durante el periodo de proliferación celular activa y que esta reducción en el número de células también es permanente. Los mismos autores han encontrado marcadas diferencias en la susceptibilidad a la desnutrición en diversas regiones del cerebro y además, que cuando se combina la desnutrición



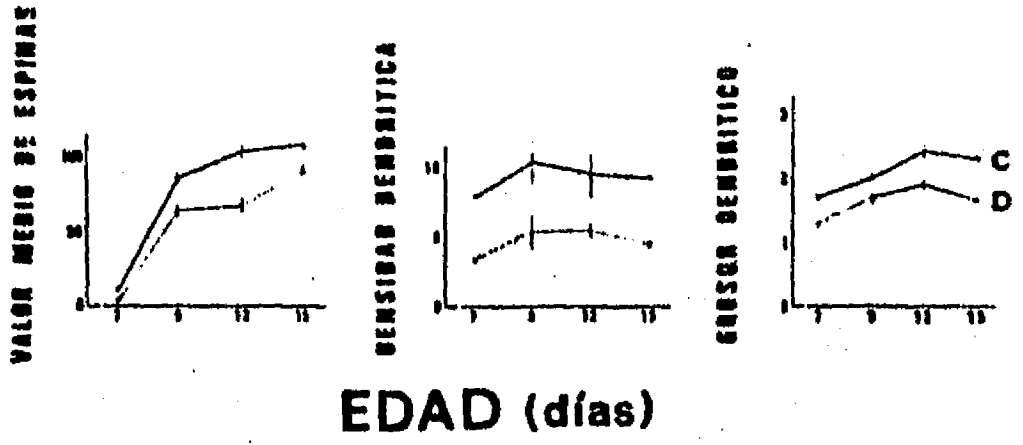


Fig. 4. Los árboles dendríticos de las neuronas piramidales de la V capa de la corteza cerebral (esquema), obtenidos de animales desnutridos durante el periodo neonatal, presentan una reducción sugnificativa en el valor medio de sus espinas, en la densidad y en el grosor dendríticos durante el desarrollo con respecto a sus testigos. El daño provocado por la desnutrición, afecta mayormente la zona del árbol dendrítico que recibe a la proyección inespecífica, comparada con la callosa y la específica. Modificado de M. Salas. Dev. Neurosci., 3: 109-117, 1980 ; M. Salas y col., Brain Res., 73: 139-144, 1974.

prenatal y la postnatal, se produce una reducción mayor en el número de neuronas que cuando se provocan separadamente.

Desnutrición y contenido de ácidos nucléicos

Por estudios de tipo bioquímico se ha encontrado que normalmente la cantidad total de ADN en el cerebro de la rata se incrementa progresivamente durante el periodo prenatal, alcanzando una meseta transitoria al nacimiento (Zamenhof y col., 1968). Este efecto parece ser uno de los parámetros de desarrollo más constantes, que a menudo se ha tomado como un indicador aproximado de la población total de neuronas generadas durante el desarrollo ontogenético. Esta correlación ha derivado en observaciones de microscopía de luz que sugieren que en la rata recién nacida, la corteza cerebral presenta predominantemente neuroblastos (Brizzee y col., 1964). Estudios posteriores han mostrado que la desnutrición de la rata antes y durante el embarazo, reduce significativamente el contenido de ADN cerebral de la rata recién nacida (Zamenhof y col., 1968) y ésto se ha interpretado como un reflejo en la reducción de la población final de neuronas. Sin embargo, dado que en los especímenes empleados para el análisis del contenido de ADN, se incluye además de las neuronas a la substancia blanca y a otros tipos celulares como la glía y las células endoteliales de los vasos sanguíneos, el contenido de ADN puede considerarse como un parámetro poco preciso para inferir acerca de la población total de neuronas.

Respecto a la posibilidad de que la reducción en la cantidad de neuronas que se aprecia en el animal desnutrido (Bass y col., 1970; Cragg, 1972; Lewis y col., 1975), pudiera determinar una alteración del SNC, la mayoría de los estudios al respecto concuerda en señalar que en estos casos, si existe una alteración de dicho funcionamiento, aunque en ocasiones sea difícil demostrarlo (Dobbing, 1972). Esta situación es congruente con el hecho de que aún se desconoce cual es el número mínimo de elementos neuronales que se requiere para realizar adecuadamente una función. Así, por ejemplo en monos infectados experimentalmente con el virus de la

poliomielitis no se altera la función motora no obstante que en estos casos, se destruye hasta en un tercio la población normal de las motoneuronas correspondientes a las extremidades inferiores. En el gato, el síndrome de espasticidad agudo provocado por la lesión de la corteza sensitivomotora, ya es imperceptible a los 3 meses posteriores a la lesión y este hecho podría explicarse sobre la base del establecimiento de un efecto plástico compensador. Sin embargo, es posible que el establecimiento de las ramificaciones dendríticas y de las conexiones sinápticas durante el crecimiento neuronal, sea más importante que el considerar sólo el número de neuronas.

Mediante estudios realizados con técnicas citoquímicas, se ha mostrado que en condiciones normales hay una intensa acumulación de ARN y de proteínas en las motoneuronas del cuerno anterior de la médula espinal de la rata, que comienza durante la vida fetal y continúa después del nacimiento (Sourander y col., 1974). Esta acumulación alcanza su mayor grado en el periodo postnatal comprendido entre los 5 y los 15 días de edad. En cambio en los animales desnutridos el peso seco y el contenido de ARN de las motoneuronas espinales, permanece significativamente reducido. En todas las ratas desnutridas, estudiadas a diversas edades, el valor del contenido de ARN decrece un 50% más que el correspondiente al peso seco de las motoneuronas en las ratas rehabilitadas, que muestra valores ligeramente inferiores a los de la ratas normales; en cambio, el contenido de ARN permanece notoriamente reducido (Haltia, 1970). Según ésto hay un efecto neuronal permanente de significado aún no bien comprendido.

De todas estas experiencias se desprende que la desnutrición llevada a cabo durante las 3 primeras semanas de la vida, causa un daño permanente al SNC, que contrasta con el poco o nulo efecto producido en edades posteriores a este periodo. En esta última condición, cuando cesa la denutrición, el cerebro es capaz aparentemente de recuperarse del daño histológico y bioquímico si el peso corporal se restituye (Sourander y col., 1974). De estos hallazgos puede concluirse que mientras más temprano en el desarrollo se establece la desnutrición, los efectos son

más severos y las oportunidades para la recuperación morfológica y funcional son menores.

Efectos de la desnutrición perinatal sobre el desarrollo de los nervios periféricos

Mielinización

Recientemente se ha corroborado mediante estudios de microscopía electrónica que la desnutrición neonatal en la rata, provoca un retraso en el depósito de la mielina, un decremento en el diámetro de los axones (Sima y Sourander, 1974 b) y un retraso en el desarrollo de las vainas perineurales de nervios periféricos (Sima y Sourander, 1973,1974 a). En términos generales la mielinización del nervio ciático se retrasa más que el crecimiento circunferencial del mismo. El retraso en el proceso de la mielinización causado por la falta de alimento de los 4 a los 12 días de edad, es aún evidente a los 26 días pero no a los 34 días postnatales, mientras que el retraso en la mielinización, después de una desnutrición de los 4 a los 18 días de edad, persiste hasta los 46 días postparto. Se ha mencionado que el progresivo crecimiento axonal, es uno de los factores que controlan la formación de la vaina de mielina (Friede y Samorajski, 1967). Sin embargo, se ha descrito que a los 12 días de edad, en el nervio ciático de ratas desnutridas, los axones de 3 a 7 micras de diámetro no guardan relación con el grosor de la vaina de mielina (Clos y Legrand, 1970). En el presente se desconoce cual pudiera ser el significado funcional de estos hallazgos, para la transmisión sensorial en etapas críticas del desarrollo.

Diversos estudios han confirmado que en el animal normal, participan las células de Schwann en el depósito de la mielina en los nervios perféricos y asimismo, que sus mitosis continúan por lo menos hasta los 6 días postnatales (Dickerson y col., 1967). En cambio, en ratas desnutridas desde el nacimiento hasta los 12 días de edad, las células de Schwann se fragmentan y pierden la organización de su reticuloendoplasma granular (Clos y Legrand, 1970). Estos hallazgos sugieren

que la desnutrición puede reducir el número de las mitosis de estas células y consecuentemente interferir con el depósito de la mielina. Sin embargo, en experimentos similares llevados a cabo en conejos, esta posibilidad no pudo confirmarse (Hedley-White, 1973). Las evidencias obtenidas en experimentos acerca de la denervación de fibras del músculo sóleo, han mostrado que las células de Schwann desempeñan un papel relevante al hipertrofiarse y permitir a través de sus procesos, que colaterales de los pies terminales de las fibras nerviosas vecinas intactas, se guien hacia las placas musculares denervadas, para propiciar su reinervación (Son y Thompson, 1995). Sin embargo, se desconoce la relevancia que estos hallazgos puedan tener, en relación a los efectos de la desnutrición neonatal para el crecimiento neural.

Desarrollo del diámetro axonal

Con el propósito de investigar si la desnutrición temprana afecta el desarrollo de los nervios periféricos, se ha estudiado la distribución de la frecuencia de los diversos diámetros de los axones, a diferentes niveles del SNC (Sima y Sourander, 1974 b; Sourander y col., 1974). En el nervio clático por ejemplo, se ha encontrado un notable retraso en el desarrollo del grosor de las fibras de gran diámetro y de las fibras delgadas, aunque no en la misma proporción. Normalmente, la distribución del calibre de las fibras del nervio ciático alcanza una configuración bimodal a los 15 días de edad y a los 30 días, cambia a una configuración trimodal. En las ratas desnutridas durante el periodo perinatal, la aparición de la configuración bimodal sufre un retraso en su aparición de 10 días aproximadamente y además, no se llega a presentar la configuración trimodal característica del animal normal. Si las ratas son rehabilitadas nutricionalmente, el estudio de sus nervios periféricos muestra una restitución en el calibre de las fibras nerviosas correspondientes (Sourander y col., 1974).

Desarrollo de las raíces de la médula espinal

Mediante el estudio del calibre de las raíces de la médula espinal de la rata, se ha visto que la desnutrición retrasa más el crecimiento de las raíces dorsales que el de las ventrales (Sourander y col., 1974). La rehabilitación nutricional llevada a cabo en varias edades del desarrollo hasta los 90 días, muestra que el calibre de las fibras se recupera en mayor grado en las raíces ventrales. Asimismo, la evaluación estadística de las mediciones, indica que la rehabilitación no restaura el calibre de las raíces dorsales de los animales previamente desnutridos. A partir de estos estudios puede decirse que la desnutrición impide el aumento del diámetro en las fibras nerviosas mielinizadas en diversos niveles del sistema nervioso. El significado funcional de estos cambios estructurales queda aún por aclararse. Sin embargo, es posible que estas alteraciones modifiquen la transmisión de los impulsos nerviosos desde la periferia que, según se señaló previamente, desempeñan un papel importante en el desarrollo de la conectividad neuronal con la consecuente alteración de la funciones integrativas del SNC.

Desarrollo de las vainas perineurales

Estudios previos han mostrado que los nervios periféricos están protegidos contra diversos agentes nocivos mediante fibras elásticas que conectan las células endoteliales de los capilares sanguíneos con las láminas celulares internas de las vainas perineurales que circundan a los nervios periféricos (Feng y Lieu, 1949; Thomas, 1963; Olsson y Reese, 1971; Key y Retzius, 1976). Estas fibras son importantes componentes de la organización estructural del sistema nervioso (Kristensson y Olsson, 1973; Sima y Sourander, 1974 a). La idea de que la desnutrición ocurrida durante etapas tempranas del desarrollo pudiera alterar la integridad de estas barreras protectoras y facilitar así la entrada de gérmenes del tipo del los virus, fue inicialmente sugerida por Sabin (1941). En los animales adultos normales, la barrera perineural es efectiva para impedir la difusión de substancias macromoleculares fluorescentes y de las proteínas marcadas aplicadas a la

superficie de los nervios expuestos (Sima y Sourander, 1974 a, b). Esta barrera está ausente en la rata recién nacida y no aparece sino hasta las tres semanas de edad. Si se emplea albúmina sérica marcada con azul de Evans, y se aplica por 24 horas en la superficie del nervio ciático de una rata normalmente nutrida y menor de 4 semanas, se observa que la albúmina penetra al perineuro y se difunde en los espacios endoneurales. El mismo fenómeno se observa en las ratas prenatalmente desnutridas mayores de 4 semanas. En los animales desnutridos no se desarrolla aparentemente ninguna barrera a la difusión de proteínas exógenas, aún durante el periodo de observación de 4 meses (Sima y Sourander, 1973). Esto abre la posibilidad de que en los organismos desnutridos haya potencialmente una vía de entrada a los gérmenes, lo cual explicaría en gran parte su gran susceptibilidad a las infecciones (Sima y Sourander, 1974 a).

Efectos sobre los mecanismos del control aferente

En los mamíferos es un hecho establecido que la aplicación iterativa de estímulos sensoriales provoca inicialmente la reacción o reflejo de orientación hacia la fuente de estimulación y posteriormente el fenómeno de la habituación (Hernández-Peón, 1956; Hernández-Peón, 1957; Guzmán-Flores y col., 1962). Durante la reacción de orientación el individuo se moviliza hacia el sitio del estímulo, mueve sus orejas, busca con la mirada y olfatea cualquier señal que le indique la localización y el carácter de la estimulación. Si se registra la actividad eléctrica cerebral, el EEG se desincroniza y los potenciales sensoriales provocados, poco o no relacionados con la fuente de la estimulación, reducen notoriamente su amplitud. En cambio en la vía sensorial relacionada con la estimulación que desencadenó la respuesta de orientación, los potenciales eléctricos provocados incrementan su amplitud en todos sus relevos sinápticos.

El fenómeno de la habituación, se inicia gradualmente conforme transcurre la aplicación repetitiva de estímulos sensoriales. Durante este proceso el sujeto muestra gradualmente indiferencia progresiva hacia los estímulos, se recuesta en el

piso, e inclusive llega a conciliar el sueño. El registro de la actividad eléctrica cerebral muestra sincronización en el EEG, actividad de somnolencia o bien de sueño. En cuanto a los potenciales eléctricos provocados, éstos reducen gradualmente su amplitud en todos los relevos sinápticos e incluso llegan a desaparecer. En la vía sensorial involucrada con el fenómeno de la habituación, los potenciales eléctricos provocados van reduciendo gradualmente su amplitud y su desaparición ocurre primero en aquellos relevos sinápticos alejados de la fuente de estimulación y después en los que están más cercanos a la misma. En cuanto a los mecanismos electrofisiológicos que participan en estos fenómenos cerebrales, se sabe que ellos tienen un carácter modulador o bien facilitador de las señales eléctricas que ascienden bajo la forma de patrones espaciotemporales en las vías sensoriales en ruta hacia la corteza cerebral.

En los últimos años nuestro grupo de investigación ha comprobado que la desnutrición neonatal, provoca un deterioro significativo en el desarrollo neuronal de aquellas estructuras que participan en la modulación de las señales sensoriales. Así las neuronas de los núcleos inespecíficos del tálamo como el núcleo reticular lateral, reuniens y medial, reducen el tamaño de sus árboles dendríticos, el número y la extensión de sus ramas y del área del pericárion. Estas alteraciones en cambio son mínimas en el caso de las neuronas de núcleos específicos del tálamo del tipo de los geniculados lateral y medio (Salas y Torrero, 1980; Salas y col., 1986). En estudios más recientes empleando el mismo modelo de desnutrición en la rata, hemos identificado que ocurre un efecto similar en las neuronas bipolares del núcleo medial de la oliva superior del tallo cerebral (Salas y col., 1994) y de las neuronas multipolares del claustrum (Escobar y Salas, 1995). Todas las estructuras antes mencionadas, forman parte de circuitos neuronales diversos que en distintos niveles del SNC, modulan el ingreso de las señales neurales sensoriales generadas en la periferia.

De la evidencia experimental antes mencionada, resulta claro que la transmisión de la información sensorial se encuentra modulada por una red neuronal

extraordinariamente compleja, de manera que los hallazgos que indican la existencia de una reducción significativa en el tamaño neuronal, del campo dendrítico y del número de ramas dendríticas neuronales en distintas edades del desarrollo postnatal, puede ser relevante para la comprensión del proceso de la transmisión sensorial específica e inespecífica del cerebro tempranamente dañado.

Debido a que el campo dendrítico y el número de sus procesos están reducidos en los animales desnutridos, particularmente para el caso de los núcleos inespecíficos, podría inferirse que la capacidad de estas estructuras para recibir relevar y modular el ingreso sensorial, podría verse trastornada. Por otro lado, de diversos estudios se sabe que las conexiones de los axones aferentes sensoriales con las células de los núcleos sensoriales, son de tal naturaleza, que constituyen un substrato básico para la integración de patrones temporales de información ascendente (Scheibel y Scheibel, 1967a). El hallazgo de que la reducción neonatal del aporte de alimento afecta las neuronas de relevo sensoriales de los núcleos inespecíficos más que las neuronas de los específicos, ofrece apoyo adicional a la hipótesis de que las influencias nocivas perinatales ejercen un mayor efecto sobre los circuitos polisinápticos, comparados con los oligosinápticos (Salas y Cintra, 1973 b; Forbes y col., 1975; Escobar y Salas, 1993; Escobar y Salas, 1995).

Estos resultados reflejan la gran vulnerabilidad de las neuronas en crecimiento a las diversas influencias perinatales. En efecto, la desnutrición neonatal aparentemente afecta más el crecimiento neuronal cuando ocurre en un momento crítico de su desarrollo, conduciendo así a un efecto final de reducción en el contenido de las sinápsis neuronales y de su actividad global desencadenada por los impulsos nerviosos.

Tomando en cuenta que la maduración del tejido cerebral puede considerarse como el producto de la interacción de varias influencias que contribuyen al desarrollo de la plasticidad que se ve en el cerebro adulto, es posible entonces que cuando uno de éstos se altera, como parece ser el caso de la nutrición deficiente, dicha

influencia modifica el desarrollo cerebral. Así este efecto, pudiera impedir en el recién nacido la percepción y manejo adecuados de los estímulos ambientales que son necesarios para su propio crecimiento neural.

ESTUDIO ELECTROFISIOLOGICO

El efecto producido por la desnutrición neonatal sobre el tejido nervioso en desarrollo, también se ha valorado mediante el análisis de la actividad eléctrica que el mismo genera. Dos son los tipos de actividad que esencialmente se han estudiado: la actividad eléctrica espóntanea de la corteza cerebral (EEG y ECoG) y el registro de la actividad eléctrica sincronizada y provocada por la aplicación de estímulos sensoriales.

Desarrollo del ECoG

El estudio de la maduración del ECoG se ha llevado a cabo en diferentes especies y condiciones experimentales, en las cuales se ha intentado establecer una estrecha correlación entre los fenómenos eléctricos y el desarrollo de las estructuras nerviosas corticales y subcorticales, y de los procesos bioquímicos que en ellas ocurren a lo largo del desarrollo (ver Purpura, 1962; Anokhin, 1964b; Ellingson y Rose, 1970).

Se ha descrito que en la rata antes de los 5 primeros días de edad, no existe en la mayoría de los casos actividad eléctrica cortical detectable y que en otros, la escasa actividad de ondas lentas que puede registrarse, se piensa que es una propagación de la actividad generada en estructuras subcorticales del tallo cerebral y del diencéfalo que poseen un mayor grado de maduración. A la edad de 5 a 6 días, el ECoG está representado por prolongados periodos de ondas lentas de muy bajo voltaje y larga duración. Sin embargo, después de los 6 días de edad, el ECoG se hace regular y se caracteriza por la presencia de ondas lentas de bajo voltaje y baja

frecuencia (60-80 μv y 2-10 cps respectivamente). Estas ondas van modificando sus características eléctricas rápida y progresivamente hasta la edad de 15 días, en que ya son muy semejantes a las del animal adulto (80-150 μv y 6-28 cps). De ahí en adelante el voltaje y la frecuencia se modifican muy poco, alcanzándose los valores del animal adulto entre los 25 y 30 días de edad aproximadamente (80-200 μv y 8-31 cps) (Deza y Eidelberg, 1967).

Desnutrición y desarrollo del ECoG

En experimentos llevados a cabo en nuestro laboratorio (Salas y Cintra, 1975), hemos observado que las ratas privadas parcialmente de alimento durante las primeras dos semanas de vida, muestran un retraso de 1 a 3 días con respecto a sus testigos en la aparición de la actividad del ECoG. En estos animales los periodos de silencio eléctrico son más prolongados y las ondas de menor voltaje que en el caso de los animales testigo. A los 7 días de edad, la actividad se hace consistente aunque el voltaje y la frecuencia de la actividad eléctrica aún son inferiores a los del animal normal. Después de los 16 días de edad, es difícil por la simple observación de los registros, apreciar diferencias entre los trazos de los animales control y los correspondientes a las ratas desnutridas.

Midiendo la frecuencia promedio del ECoG de las ratas testigo y de las ratas desnutridas durante el desarrollo postnatal, hemos encontrado que desde las primeras edades estudiadas, los animales desnutridos presentan una mayor proporción de ondas lentas y asimismo, que esta diferencia generalmente tiende a desaparecer hasta los 16 días de edad (Salas y Cintra, 1975). Durante la edad adulta el ECoG correspondiente a las áreas frontal y occipital de la corteza cerebral, no muestra diferencias significativas en cuanto a la distribución de sus frecuencias. Sin embargo, en el área temporal existe un predominio significativo de la actividad lenta (Fig. 5). Los resultados de este estudio están acordes con aquellos reportes que sugieren que la falta de un aporte adecuado de nutrimentos durante las estapas

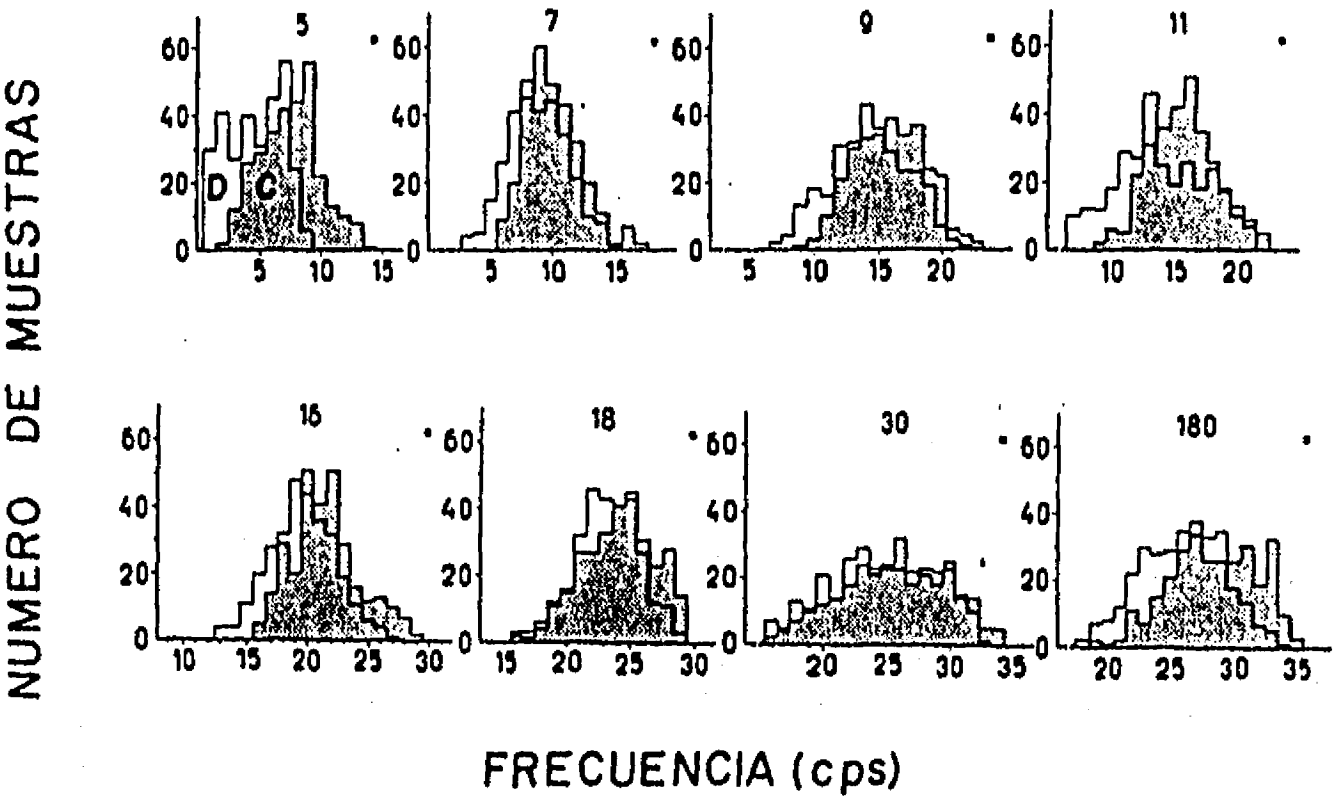


Fig. 5. Histogramas de frecuencia promedio del ECoG, obtenidos de la corteza temporal de ratas normales y desnutridas durante el desarrollo neonatal. Nótese que en el grupo desnutrido (D), hay un predominio de las frecuencias lentas cuando se compara con los histogramas de sus controles (C) correspondientes. Modificado de M. Salas y L. Cintra. Physiol. Behav., 14: 589-593, 1975.

iniciales del desarrollo, afecta el patrón de maduración de los ritmos espontáneos electrocorticales (Engel, 1956; Nelson, 1959; Nelson y Dean, 1959).

Diversos estudios morfológicos y electrofisiológicos, han establecido la especificidad de las conexiones sinápticas a una zona restringida de la neurona (Andersen y col., 1963, Blackstad y Flood, 1963). Así en las células piramidales grandes de la capa V de la corteza cerebral, las sinapsis excitatorias se localizan principalmente en las prolongaciones dendríticas y sus espinas; mientras que las conexiones inhibitorias se localizan en la base de las dendritas basales y en el cuerpo neuronal (Eccles, 1964; Purpura y col. 1965). En otros estudios hemos observado que en ratas desnutridas durante la edad temprana, hay una marcada reducción en el número total de los procesos espinosos, sin alterarse aparentemente la proporción de los diferentes tipos de espinas (Salas y col., 1974; Salas, 1980). Estos hallazgos, pueden explicar parcialmente el predominio de las frecuencias lentas en las ratas desnutridas, debido posiblemente a una reducción en la proporción de las conexiones sinápticas tanto excitatorias como inhibitorias. Esta posibilidad adquiere aún mayor significado, si se considera que el ECoG se genera principalmente por un proceso de sumación de los potenciales eléctricos de las dendritas apicales y en parte, por los potenciales de campo propagados desde las prolongaciones basales (Purpura, 1962). Por otra parte la persistencia de las ondas lentas en el electrocorticograma de la corteza temporal de las ratas desnutridas, apoya la idea de que esta estructura es particularmente sensible durante el periodo neonatal, a los efectos nocivos producidos por la falta de alimento (Taori y Pereira, 1974; Salas y Cintra, 1975). Asimismo, estos resultados podrían tener relación con un estudio electrofisiológico previo que muestra que las ratas que son desnutridas durante las tres primeras semanas de la vida, presentan una marcada susceptibilidad a las crisis convulsivas provocadas por la estimulación eléctrica (Stern y col., 1974; Forbes y col., 1978).

Desarrollo de los potenciales eléctricos provocados

Los potenciales eléctricos provocados por la aplicación de estímulos sensoriales debido a su relativa constancia y a su dependencia de la intensidad y frecuencia de la calidad de la estimulación, se han empleado frecuentemente para explicar la organización y funcionamiento del substrato neural, la naturaleza de los potenciales cerebrales en general y la generación de la actividad eléctrica de la corteza cerebral en particular.

Se ha sugerido que durante la ontogenia neural, los potenciales provocados se generan sobre la base de un doble mensaje de activación ascendente (Anokhin, 1964 b). El primer mensaje está formado por impulsos que se propagan a través de una vía de conducción larga, que madura relativamente pronto y que envía sus fibras directamente a la capa plexiforme y a las dendritas apicales de las neuronas corticales. Un ejemplo típico de estas vías lo constituye la radiación talámica inespecífica (Nauta, 1954; Purpura, 1962; Anokhin, 1964 b). Los impulsos del segundo mensaje, viajan a lo largo de una vía corta de maduración lenta que termina fundamentalmente en la base de las dendritas apicales, dendritas basales y en el soma neuronal de las células piramidales de la capa IV cortical. Dentro de este tipo de vías se encuentra la radiación talámica específica (Anokhin, 1964b; Nauta, 1954; Globus y Scheibel, 1967 b; Szentágothai, 1969) (Fig. 6).

Efectos sobre los componentes de la respuesta eléctrica

El daño provocado por la desnutrición neonatal sobre las neuronas en desarrollo, se ha valorado mediante el empleo de la técnica de los potenciales provocados. De esta manera se ha reportado que como resultado de la desnutrición, los componentes primarios de los potenciales provocados en las áreas somestésica, visual y auditiva de la corteza cerebral, alargan su latencia y reducen su amplitud (Mourek y col., 1967; Callison y Spencer, 1968; Salas y Cintra, 1973b). Sin embargo, estos cambios van desapareciendo progresivamente hasta que a los 16 días de

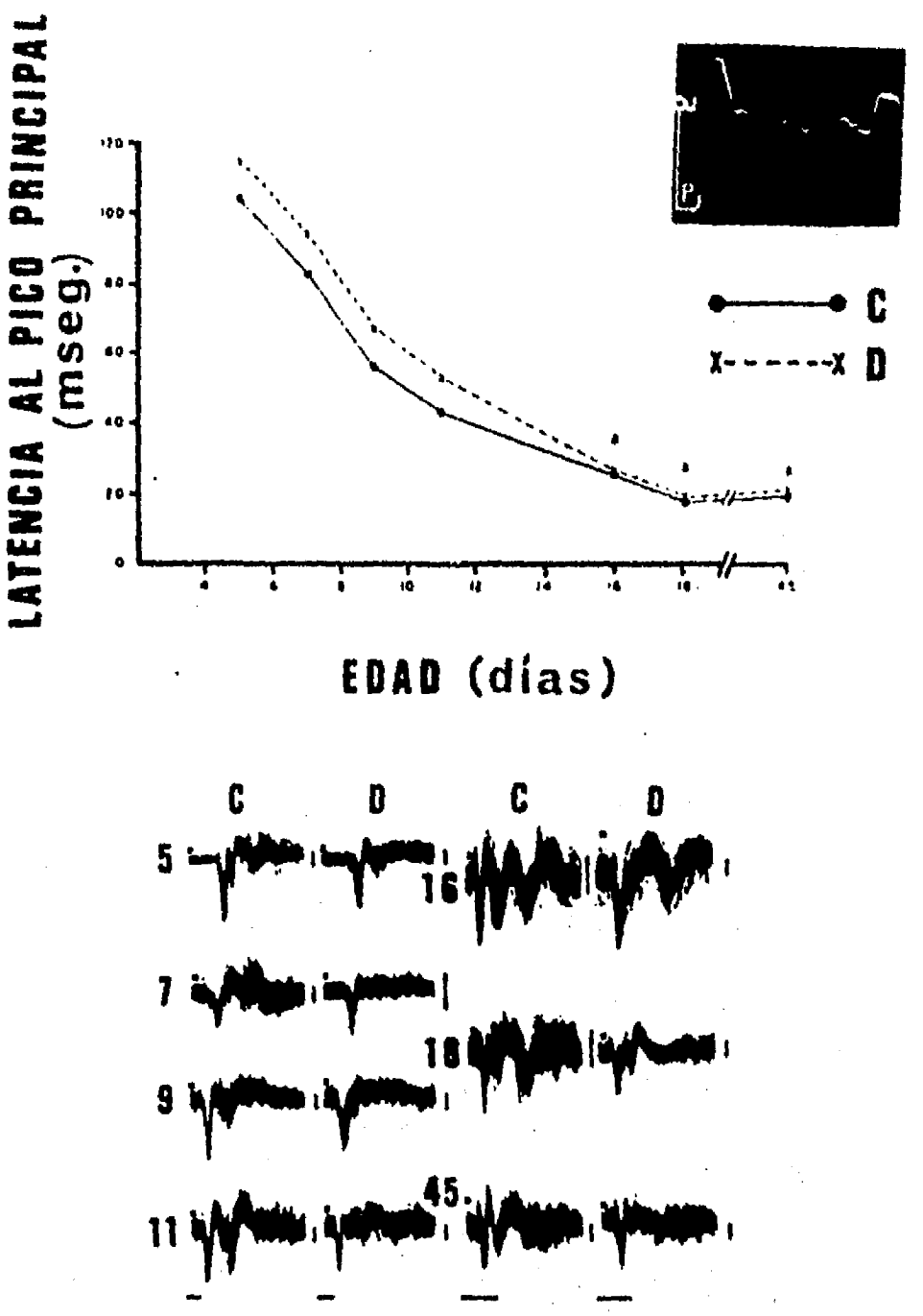


Fig. 6. Latencias al pico principal de los potenciales provocados en el área sensitivomotora de la corteza cerebral de ratas normales (C) y desnutridas (D) durante el desarrollo, por la aplicación de estímulos eléctricos umbral al nervio ciático contralateral. En la parte inferior puede observarse que cuando se superponen 10 respuestas sucesivas, en los animales desnutridos hay una marcada inconsistencia en la presencia y configuración de los componentes tardíos de los potenciales provocados. Calibraciones, 100 mseg y 50 μv. Modificado de M. Salas y L. Cintra. Physiol. Behav., 14: 589-593, 1975.

edad no difieren de los correspondientes a los animales testigo. Estos cambios en los componentes primarios del potencial provocado, se han tratado de explicar como una consecuencia del retraso en el proceso de la mielinización, de la reducción del diámetro axonal de las aferentes sensoriales y de las alteraciones en la conectividad cortical a nivel del sistema inespecífico (Salas y Cintra, 1973b; Sima, 1974 b; Salas, 1980; Fuller y Wiggins, 1984).

En un estudio electrofisiológico realizado en nuestro laboratorio (Salas y Cintra, 1973b), hemos visto que además de estos efectos en los componentes primarios, los potenciales secundarios de larga latencia que siguen a los componentes primarios decrecen en su número y amplitud. Este hecho sugiere que el sistema de proyección inespecífico involucrado en la génesis de los potenciales secundarios, aparentemente se halla más dañado por la desnutrición que el sistema específico, relacionado a su vez con el origen de las respuestas primarias de latencia corta (Lindsley, 1970). Es de interés hacer notar, que los efectos de la desnutrición neonatal sobre el sistema de proyección inespecífico, repercuten en mayor grado sobre el desarrollo del SNC, que los efectos de la desnutrición sobre el sistema específico. Esta observación es muy significativa desde el punto de vista funcional, dado que está bien establecido que el sistema inespecífico juega un papel fundamental en el origen de los estados de conciencia, de la atención selectiva, la percepción y la conducta emocional, así como en el control de los ritmos eléctricos neocorticales (Lindsley, 1970). Siguiendo esta idea es posible que la baja capacidad de aprendizaje (Barnes y col., 1966; Simonson y Chow, 1970; García-Ruíz y col., 1994), las alteraciones del ciclo sueño vigilia (Jacobs y Mc Ginty, 1971; Cintra y col., 1988) y los trastornos de la conducta emocional que se presentan en las ratas adultas durante la privación de alimento (Franková, 1973 a y b), pudieran ser debidos a las alteraciones que ocurren principalmente en el sistema de proyección inespecífico. Esta interpretación se ha confirmado por otros estudios morfológicos y electrofisiológicos posteriores (Forbes y col., 1975; Salas y Torrero, 1980; Escobar y Salas, 1993).

Efectos sobre la variabilidad y la duración de la postdescarga electrocortical

En observaciones electrofisiológicas preliminares sobre la variabilidad y la duración de las postdescargas provocadas en la corteza cerebral por la estimulación sensorial, hemos comprobado que los animales desnutridos desde el nacimiento hasta los 25 días de edad, presentan mayor variabilidad y duración de las ondas repetitivas que aquellos animales que no fueron desnutridos. Estos hallazgos confirman nuestras observaciones anteriores que nos han llevado a sugerir la existencia de una deficiencia en el desarrollo de los mecanismos cerebrales inhibitorios y excitatorios (Salas y Cintra, 1975), como la causa del predominio de las ondas lentas en los animales desnutridos. Además esta hipótesis concuerda con estudios previos realizados en niños retrasados mentales en los que se ha mostrado que algunos componentes de los potenciales provocados, aumentan su amplitud como consecuencia de un desarrollo insuficiente de posibles mecanismos inhibitorios (Barnet y Lodge, 1967; Ornitz y col., 1968).

ESTUDIO CONDUCTUAL

De enorme importancia ha sido conocer los efectos que la desnutrición perinatal ejerce sobre el desarrollo de la conducta, y sobre todo de los efectos que ejerce a largo plazo sobre las funciones complejas que implican grandes cambios plásticos en el SNC.

Dos son los tipos de patrones conductuales más estudiados experimentalmente sobre los cuales influye la desnutrición neonatal: (a) la conducta refleja y (b) los procesos conductuales complejos del tipo de la memoria, la atención selectiva, la habituación, el aprendizaje, etc.

Conducta refleja

Efectos sobre diversos patrones reflejos

En estudios relacionados con la conducta refleja de la rata, se ha puesto en claro que la desnutrición perinatal produce un notable retraso en el desarrollo de dichos patrones (Altman y col., 1970; Salas, 1972; Salas y Cintra, 1973 a; Salas y col., 1991). Así los efectos de la desnutrición se han valorado mediante el empleo de diversos tipos de actividad refleja tales como la deambulación, los reflejos de enderezamiento, los movimientos de pivoteo, el desarrollo del husmeo y otras actividades motoras provocadas en el animal experimental como es inducirlo a colgarse y moverse sobre una cuerda horizontal, a sujetarse y descender por cuerdas de diferentes calibres, etc. También se ha valorado la capacidad de las ratas infantes para regresar al nido y para la locomoción (Altman y col., 1970, 1975; Clarke y col., 1992; Gramsbergen y Westerga, 1992).

Tanto en los animales testigo como en los desnutridos perinatalmente, los reflejos de enderezamiento no se presentan antes del quinceavo día de edad. Sin embargo, en los animales desnutridos disminuye en forma significativa la amplitud de estos reflejos. Con respecto a movimientos oscilatorios de la extremidad cefálica (pivoteo), los animales desnutridos reducen significativamente esta actividad hasta el décimo día de edad. Este decremento de los movimientos de pivoteo persiste 3 ó 4 días más, que en los animales testigo. En relación con el husmeo, tanto los animales testigo como los desnutridos, incrementan progresivamente esta actividad hasta los 11 y 12 días postnatales. De esa edad en adelante, las ratas desnutridas presentan una disminución significativa que persiste hasta los 15 ó 16 días de edad (Altman y col., 1970).

En relación con la actividad motora provocada, se ha descrito que entre los 5 y 11 días de edad los animales desnutridos disminuyen significativamente su capacidad para permanecer colgados en una cuerda horizontal en relación con los

animales testigo. Esta diferencia desaparece entre los 15 y 20 días de edad. La medición de la habilidad para trepar o para descender por una cuerda colocada verticalmente, indica que después de la primera semana de edad, los animales desnutridos tienden a sujetarse durante periodos más largos que los animales testigo. Esta diferencia desaparece a los 25 días de edad. A partir del veinteavo día de edad, los animales desnutridos presentan un decremento significativo de la capacidad para trepar por una cuerda vertical y esta diferencia persiste hasta los 26 días de edad. Finalmente, los animales desnutridos emplean significativamente mayor tiempo en regresar al nido y presentan alteraciones en la locomoción y en el desarrollo muscular en comparación con los animales normalmente nutridos, esta diferencia persiste hasta el día 21 y 22 de edad (Altman y col., 1970). Paralelamente, se conoce de la persistencia de alteraciones en la locomoción asociadas al daño de motoneuronas espinales y al desarrollo de los músculos esqueléticos (Altman y Sudarshan, 1975; Bedi y col., 1982; Clarke y col., 1992; Gramsbergen y Westerga , 1992; Wilson y col., 1988; Westerga y Gramsbergen, 1990).

Desarrollo del patrón reflejo de nado

Estudios previos en nuestro laboratorio han establecido que el desarrollo de la conducta rafleja de nado, constituye un modelo experimental adecuado para valorar los efectos producidos por diversas influencias perinatales sobre la maduración del SNC (Salas, 1972; Salas y Cintra, 1973 a). En un estudio previo (Schapiro y col., 1970), observamos que de los diversos patrones de movimiento que los animales realizan durante la actividad de nado, los cambios en la posición de la cabeza con respecto a la superficie del agua y los movimientos de los miembros anteriores resultan ser los más factibles de ser medidos.

Normalmente la rata, antes del quinto día de edad, es incapaz de nadar. A esta edad el animal sólo flota, manteniéndose parcial o completamente sumergido en el agua; comúnmente presenta arqueo y torsión del dorso del cuerpo y mantiene las extremidades, cuello y cola en hiperextensión. Entre los 5 y los 15 días de edad,

mejora la habilidad para nadar, la cabeza que en un principio se mantenía bajo la superficie del agua, progresivamente se va levantando hasta que el animal la mantiene erguida sacando la nariz y parte de la cabeza fuera del agua. En relación con los movimientos de los miembros anteriores, desde los 5 a los 16 días de edad, el animal presenta movimientos vigorosos de flexión y extensión. Sin embargo, de los 17 a los 21 días de edad, estos movimientos progresivamente van decreciendo, tanto en su número como en su amplitud, hasta desaparecer. De esta edad en adelante, los miembros se mantienen en hiperextensión y sólo se mueven ligeramente para permitir cambios en la dirección del nado. Asimismo, desde el nacimiento hasta los 5 días de edad, los animales sólo flotan en el seno del agua, de los 6 a los 11 días, nadan describiendo círculos en ambos sentidos y de los 12 días en adelante, nadan casi exclusivamente en línea recta.

En las ratas normales y en las desnutridas por un periodo de 10 días, desde el nacimiento por el método de la separación parcial de las crías (12 h) en una incubadora, hemos medido los citados patrones de movimiento, durante el nado. Los resultados de este estudio indican que los animales desnutridos, en comparación con sus testigos, presentan un retraso de 2 a 3 días en el desarrollo de la habilidad para erguir la cabeza fuera del agua y en alcanzar el patrón de hiperextensión de los miembros anteriores. Además flotan y nadan en círculos durante 2 a 3 días más que los animales testigo (Fig. 7). Asimismo, experiencias aún no publicadas, sugieren que el reflejo de escape del agua hacia una plataforma, presentan un retraso de 2 a 3 días en las ratas desnutridas.

Todas estas experiencias sugieren que la desnutrición ocurrida durante la etapa neonatal, interfiere severamente con el desarrollo de las estructuras del SNC involucradas en la coordinación e integración de las diferentes actividades reflejas. Este punto de vista está de acuerdo con experiencias previas en las que se sabe que la corteza sensitivomotora, el cerebelo, el vestíbulo y posiblemente otras áreas del SNC que controlan el movimiento, son afectadas por la privación neonatal de alimento (Schapiro, y col., 1970; Bass y col., 1970; Salas, 1972).

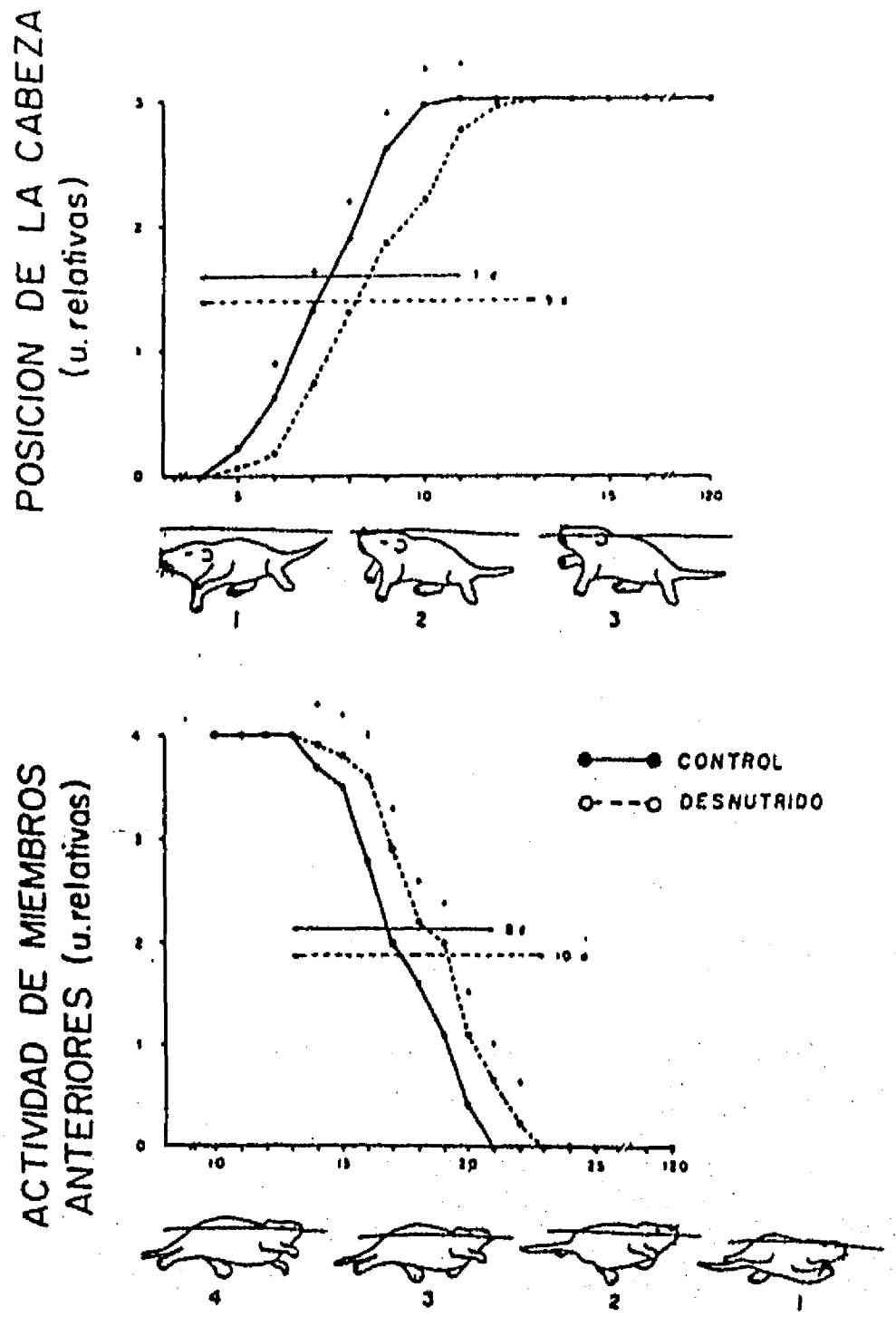


Fig. 7. Actividad de nado obtenida en ratas control y desnutridas durante el desarrollo neonatal. Tanto la capacidad para sacar la cabeza fuera del agua, como la habilidad para reducir los movimientos de flexión y extensión de los miembros anteriores, se ve retardada significativamente en los animales desnutridos. Las flechas indican el tiempo en días requerido para que se desarrollen ambos patrones conductuales en los dos grupos experimentales. Modificado de M. Salas. Physiol. Behav., 8: 119-122, 1972.

Plasticidad potencial para la conducta refleja

Con base en nuestros experimentos (Schapiro y col., 1970; Salas, 1972; Salas y Cintra, 1973a) así como en el de otros autores (Altman y col., 1970), puede afirmarse que el retraso en el desarrollo neuromuscular que se ve en el infante, es imperceptible cuando el sujeto alcanza los 30 días de edad, debido probablemente a que el propio SNC compensa el daño cerebral. Sin embargo, el hecho de no encontrarse anormalidades en el sistema neuromuscular durante la vida adulta, no excluye la posibilidad de que persistan deficiencias funcionales, que las técnicas actuales de medición de la conducta no permiten aclarar. Por esta razón y debido a que en otros procesos más complejos como la locomoción, el aprendizaje, la memoria y la conducta emocional si se manifiestan deficiencias funcionales durante la vida adulta, tiene gran interés el estudio de los cambios producidos por la desnutrición neonatal sobre estos procesos fisiológicos.

Privación neonatal de alimento y procesos conductuales complejos

Las primeras experiencias en relación con el efecto de la privación perinatal del alimento y el aprendizaje, datan del principio de este siglo (Biel, 1938, 1939). En estos estudios se sugirió que las ratas desnutridas cometían, en relación con las testigo, un mayor número de errores para alcanzar la meta cuando eran colocadas en un laberinto múltiple en forma de T. Estos resultados se han corroborado tanto en la rata como en otras especies, incluyendo al hombre (Barnes y col., 1966; Cravioto y Robles, 1965; Franková, 1973b).

Paralelamente a estos hallazgos, se ha puesto de manifiesto que la conducta aprendida puede ser modificada por diversos factores ambientales y este hecho, dificulta la interpretación de los efectos producidos por una sola maniobra experimental como es la desnutrición. Los datos de la literatura, acerca de los efectos de la privación de alimento sobre el aprendizaje muestran una gran variación, lo cual no es más que un reflejo de la multidependencia en el

determinismo de la conducta (García-Ruíz y col., 1994). Así por ejemplo, se ha encontrado que no existe ninguna correlación entre la desnutrición moderada después del destete y el grado de aprendizaje que los animales adquieren en la prueba de Hebb-Williams (Cowley y Griesel, 1963). Por el contrario, ratas macho privadas severamente de proteínas en la dieta durante los 11 días subsiguientes al nacimiento, presentan una pobre discriminación y cometen mayor número de errores durante el aprendizaje en un laberinto con agua en forma de Y (Barnes y col., 1966; Tonkiss y col., 1991). Se ha reportado también que aquellas ratas adultas que fueron alimentadas cuando infantes por madres que sufrieron severa desnutrición durante la lactancia, tardan más tiempo en iniciar y realizar la prueba de condicionamiento en un laberinto en forma de T (Simonson y Chow, 1970). De estudios similares se ha descrito que las ratas desnutridas no presentan ningún cambio en la discriminación visual o en la habilidad mostrada durante la inversión del aprendizaje (Rajalakshmi y col., 1965). En el ratón alimentado con una dieta hipoproteínica hasta antes del destete, no se modifica su capacidad para realizar el mismo aprendizaje que los animales testigo. En otro estudio se han descrito hallazgos semejantes en ratas adultas desnutridas perinatalmente (Howard y Granoff, 1968).

Durante el condicionamiento de evitación a un choque eléctrico, se ha encontrado que normalmente el número de respuestas motivadas por el "miedo" aumentó gradualmente a pesar de que la estimulación nociva sea mínima (Franková, 1973 b). Al respecto, se ha sugerido que la experiencia al estrés provocada por la primera prueba, deja una huella intensa en el SNC del animal, en tal forma que en pruebas ulteriores la sóla exposición al ambiente del condicionamiento provoca la respuesta emocional (Franková, 1973 b). Durante el proceso de condicionamiento las ratas testigo, aprenden a calcular el intervalo de tiempo entre un estímulo y otro para evitar el choque eléctrico. Sin embargo, en los animales desnutridos se altera la capacidad para realizar este tipo de condicionamiento.

Se ha señalado que las ratas desnutridas son más susceptibles a las influencias del ambiente exterior. Así, la presentación de un estímulo acústico en

estos animales, provoca una respuesta emocional mayor y una extinción más lenta en comparación con los animales testigo (Barnes y col., 1966; Franková, 1973 b). Asimismo, la familiaridad con la situación experimental parece favorecer el aprendizaje de evitación (Franková, 1973 b). En cambio en los animales testigo, aparentemente, no parece haber ninguna relación con el grado de aprendizaje que alcanza el animal, lo cual sugiere que las ratas desnutridas son más susceptibles a los cambios del ambiente circundante.

De todos estos hallazgos parece concluirse que existen diversos factores metodológicos que pueden influir sobre los resultados individuales del aprendizaje tales como el tipo de prueba experimental, la magnitud y la duración del periodo de privación de alimento, la manipulación temprana, la edad de los animales, el sexo, el horario al cual se realiza la prueba, la estimulación sensorial dada por el experimentador, etc., que deben tenerse en cuenta para valorar e interpretar adecuadamente no sólo los efectos de la desnutrición, sino también el efecto de otras influencias sobre el aprendizaje. La falta de una adecuada valoración de estos factores, posiblemente pueda explicar en algunos casos la ausencia de efectos y en otros, los hallazgos contradictorios reportados en diferentes estudios (Franková, 1973 b).

Desnutrición neonatal y desarrollo de la conducta social

El estudio de patrones conductuales complejos ha tenido un gran impulso en el curso de los últimos años, particularmente, en relación con el desarrollo de la conducta social entre miembros de pequeños y de grandes grupos (Franková 1972, 1973 a). La madre y su camada constituyen un grupo social pequeño, que a menudo se ha empleado como modelo experimental para el estudio de las influencias que modifican la conducta social.

Generalmente la conducta materna se valora: midiendo la capacidad de la rata para reunir en el nido a los miembros de su camada, intencionalmente dispersados

por el experimentador, midiendo su habilidad para construir y mantener en buenas condiciones el nido, o bien midiendo el tiempo que ocupa la madre en lamer a sus crías, en acicalarse o en amamantar a sus recién nacidos, etc.

En diversos estudios (Franková, 1972, 1974; Smart y Preece, 1973) se ha descrito que las ratas adultas que han recibido una dieta pobre en proteínas durante los últimos 6 días del embarazo, se mantienen indiferentes ante sus ratas infantes previamente dispersadas, o bien presentan una prolongada latencia para recogerlas y depositarlas nuevamente en el nido. En estos mismos casos las dimensiones del nido son pequeñas, parte del material de éste aún está disperso, pudiendo el nido en su construcción ser amorfo, o bien circular o semicircular. Asimismo, pasan mayor tiempo realizando actividades no exploratorias y escasa actividad maternal (Franková, 1971,1972). Estos efectos sugieren que la dieta reducida en proteínas, deteriora la expresión de la conducta maternal y asimismo, que la cantidad de leche producida por la madre se reduce, con lo cual se modifica también el crecimiento y la conducta del lactante y la relación social de éste con su madre.

Conducta maternal

Como se mencionó en secciones anteriores el hombre y los roedores, nacen con una inmadurez tan marcada que se requiere de una importante participación de la madre para asegurar la sobrevida del recién nacido. En los últimos años el estudio de los distintos componentes de la conducta maternal, ha tenido un enorme avance, particularmente en cuanto a la repercusiones que ésta tiene para el desarrollo de la conducta futura de los miembros de la camada. En efecto está claro que cuando la respuesta materna hacia la cría se ve interferida, entonces estas últimas muestran en el corto o en el largo plazo conductas anormales que afectan al individuo en particular o al grupo social en lo general. Por este motivo el empleo de modelos animales para el estudio de las alteraciones de conducta asociadas a la conducta maternal, reviste hoy en día una gran importancia.

La conducta maternal en los mamíferos como la rata aparece alrededor de una semana antes del parto, particularmente desencadenada por cambios de tipo endocrinológico que concurren hacia el fin del embarazo. Durante este periodo la conducta maternal se manifiesta por la construcción del nido y el incremento de autolamido dirigido hacia la región perimamaria, abdominal y perigenital. Tales cambios se ha mostrado que tienen como propósito, obtener un espacio seguro y adecuado para la protección de la madre y su futura camada. Asimismo, promover el desarrollo mamario y estimular los mecanismos que desencadenan la producción láctea (Roth y Rosenblatt, 1968).

Siguiendo al parto, a los cambios conductuales anteriormente mencionados, se asocian otros componentes de la conducta maternal que incluyen al amamantamiento o alimentación de la camada, al autolamido corporal y anogenital de la crías y al acarreo de las mismas hacia el nido. La respuesta maternal hacia los recién nacidos se establece importantemente alrededor del día cuarto postnatal, manteniéndose así hasta el día 14 postparto, después del cual declinan todos los componentes conductuales hasta cerca del día 30 postnatal. Tanto en la iniciación como en el mantenimiento y la declinación de la conducta maternal, las crías desempeñan un papel fundamental como fuente de estímulos que modifican progresivamente la respuesta maternal (Rosenblatt y col., 1988).

Los mecanismos neurohumorales específicos involucrados en el inicio de la conducta maternal aún se encuentran en estudio, existiendo en el presente evidencias experimentales que reconocen el papel esencial de la relación estrógenos- progesterona, de la prolactina, la oxitocina y las endorfinas en el control de la respuesta maternal (Rosenblatt y col., 1988). Paralelamente en otra linea de investigación se ha establecido que otras regiones del SNC que incluyen al área preóptica media, la amígdala temporal, la vía olfatoria accesoria y el complejo habenular, son relevos esenciales del substrato neuronal involucrado en la regulación de la conducta maternal (Numan y col., 1977; Del Cerro y col., 1991; Corodimas y col., 1993).

Estudios previos han mostrado que la desnutrición en la rata adulta lactante, deteriora severamente la respuesta maternal hacia las crías recién nacidas. Así, el tiempo de amamantamiento a la camada, el lamido de las crías, el acarreo de los recién nacidos hacia el nido y la construcción de este último sufren un deterioro significativo (Franková, 1972; Smart y Preece, 1973; Smart,1976; Massaro y col., 1977). En estudios posteriores se ha establecido que la desnutrición neonatal, provoca serias alteraciones de la conducta maternal en el estado adulto, valoradas por la medición del tiempo que la madre permanece alimentando a sus crías, la construcción del nido y la latencia en el acarreo de las crías previamente dispersadas. Paralelamente ocurre un incremento del autoaseo y de movimientos circulares compulsivos (Salas y col., 1984). Debe destacarse que estas alteraciones representan un efecto de largo plazo, ya que en este estudio, las madres lactantes no se encontraban ya bajo los efectos de una deficiente alimentación. Estudios complementarios han mostrado que la privación sensorial que resulta de la interferencia en la relación madre-crías, parece desempeñar un papel importante en las alteraciones a largo plazo en la conducta maternal (Salas y col., 1984). En efecto, se sabe que cuando a las crías recién nacidas desnutridas se les estimula sensorialmente a través del manoseo y de la exposición a cajas con ambiente enriquecido conteniendo juguetes, es posible atenuar las alteraciones en la conducta maternal futura de las mismas (Regalado, 1993).

A pesar de todos estos antecedentes, se desconoce por completo si la alterada conducta maternal de ratas tempranamente desnutridas, se atenúa o compensa como resultado de la experiencia dejada por partos sucesivos. Asimismo, si otros componentes de la conducta maternal como el acarreo de las crías, el lamido anogenital, el movimiento circular y el análisis de la vocalizaciones emitidas por las crías, también pudieran modificarse a través de partos sucesivos. En relación a este último punto los estudios preliminares de nuestro grupo, han mostrado que la experiencia dejada por partos sucesivos, minimiza los efectos dejados por la desnutrición temprana. Asimismo, que cuando la desnutrición neonatal se acompaña de privación sensorial, los efectos sobre la conducta maternal se corrigen poco o

bien requieren de la experiencia de más de 3 partos sucesivos para revertirse. El hallazgo de reversión de efectos, pudiera no ser el que las madres se vuelvan más eficientes, sino más bien que su respuesta de emotividad, pudiera ser menor mostrando la madre un comportamiento más relajado para atender a las demandas de sus crías.

Desnutrición, conducta y manipulación sensorial

En un estudio previo (Franková, 1972) se han descrito los efectos de la desnutrición sola, así como de la desnutrición asociada con privación sensorial durante la fase del desarrollo cerebral. De acuerdo a estos hallazgos, cuando se estudió en un campo abierto la actividad exploratoria de las ratas adultas que únicamente habían sido desnutridas, se observó un decremento de la actividad exploratoria. Este efecto fue mayor en aquellos animales que además de la desnutrición, se les había reducido la información sensorial. En otros trabajos se ha mostrado que los efectos de la desnutrición en el recién nacido, pueden compensarse mediante la aplicación de un exceso de estimulación sensorial (Franková, 1972; Levitsky y Barnes, 1972; Escobar y Salas,1987; Regalado, 1993). Estos resultados han tenido un fin práctico en el caso del hombre y así, en algunas instituciones de salud en donde se asiste a niños con retraso mental de diferentes grados, éstos son sometidos a sesiones diarias de hiperestimulación sensorial, con el propósito de mejorar su deficiencia mental. Esta práctica hospitalaria, parece ser de gran utilidad mientras más temprano se establezca como terápia rehabilitadora.

Desnutrición y conducta emocional

En experiencias de nuestro grupo de trabajo, hemos medido la respuesta emocional de ratas adultas que fueron desnutridas durante el periodo perinatal. La medición de la conducta emocional la hemos realizado mediante el conteo del número de cuadros cruzados en un campo abierto, del número de cuadros que fueron orinados, de los bolos fecales eliminados y la respuesta de inmovilización o

"congelamiento" durante 3 minutos de observación. Estas mediciones usualmente se han considerado como indicadores confiables de la respuesta simpático-adrenal, que se desencadena durante la adaptación a un ambiente novedoso (Levitsky y Barnes, 1970). Los resultados muestran que en general los animales desnutridos de ambos sexos, deambulan menos y presentan respuestas de inmovilización, defecación y micción en mayor grado que aquellos animales normalmente nutridos. Asimismo, la mayor parte del periodo de observación los sujetos lo emplean en actividades no exploratorias como el acicalamiento, el rascado, etc. Estos resultados sugieren que como consecuencia de la desnutrición neonatal, se altera la conducta emocional y la capacidad de adaptación hacia ambientes novedosos, debido posiblemente a un daño en las estructuras del sistema límbico que están involucradas en esta conducta como es el caso de la amígdala temporal y el hipotálamo. En un estudio reciente nuestro grupo de trabajo ha mostrado que las neuronas de la amígdala temporal, muestran alteraciones significativas de empobrecimiento en el desarrollo del árbol dendrítico. Así estos hallazgos, pudieran constituir parte de la base estructural para explicar las alteraciones en la conducta emocional de la rata neonatalmente desnutrida (Escobar y Salas, 1993). Al mismo tiempo, los hallazgos conductuales sugieren la posible existencia de un deterioro del eje hipotálamo-hipófisis-adrenal, que participa también en el desarrollo de los diversos componentes hormonales que integran la conducta emocional.

Los siguientes hechos experimentales dan apoyo a la hipótesis de que la desnutrición neonatal daña al eje hipotálamo-hipofisiario. En las ratas privadas de alimento se reduce el tamaño de la glándula hipófisis y su contenido de hormona de crecimiento. Esta deficiencia hormonal guarda una estrecha correlación con el enanismo que acompaña al síndrome de desnutrición en sus diversos grados (Stephen, 1940). Tanto en el hombre como en los animales desnutridos, se ha observado un marcado retraso en la maduración de los órganos reproductores, así como en la apertura vaginal y en el inicio de la pubertad (Calixto, 1994). Este último hecho también sugiere que la falta adecuada de nutrimentos, provoca una deficiencia en la secreción de las gonadotrofinas hipofisiarias (Kennedy y Mitra,

1963), o bien que señales endógenas de tipo metabólicas, causadas por la baja de peso, de la talla corporal y/o de la composición de los tejidos también pudieran ser las responsables del retardo en la maduración gonadal (Cameron, 1991).

Alteraciones en el desarrollo de la conducta de autoaseo

Dentro del amplio repertorio conductual que presentan los roedores, destaca de manera sobresaliente la conducta de autoaseo. En efecto de diversos estudios se ha establecido que durante la fase de vigilia, esta conducta ocupa alrededor del 60% con respecto a otros componentes conductuales. Este hecho y las modificaciones que sufre el autoaseo en diferentes estados funcionales, revela la gran variedad de efectos que promueve este comportamiento.

En este contexto se ha establecido que el autoaseo, desempeña un papel central en la eliminación de ectoparásitos de la piel de los flancos, del vientre, de la cabeza y de las extremidades lo cual permite mantener la limpieza y lozanía de la piel (Borchelt y col., 1976; Patenaude y Bovet, 1983). Por otro lado se conoce que tiene un papel importante para permitir la eliminación de energía calórica, ya que dentro de cierto límite el humedecimiento de la piel a través del lamido libera calor al medio ambiente por radiación y evaporación, permitiendo así regular la temperatura (Hainsworth y Stricker, 1970). En otros estudios se ha reconocido que el autolamido tiene un papel promotor del desarrollo gonadal en animales prepúberes (Moore y Rogers, 1984), del crecimiento mamario y de su secreción en los últimos días del embarazo de la rata (Roth y Rosenblatt, 1968). Asimismo, el autolamido se incrementa cuando los sujetos son expuestos a un ambiente novedoso, extraño o estresante (Jolles y col., 1979; Gispen y col., 1988) y también se incrementa por razones desconocidas durante el proceso del envejecimiento en la rata (Kametani, 1988).

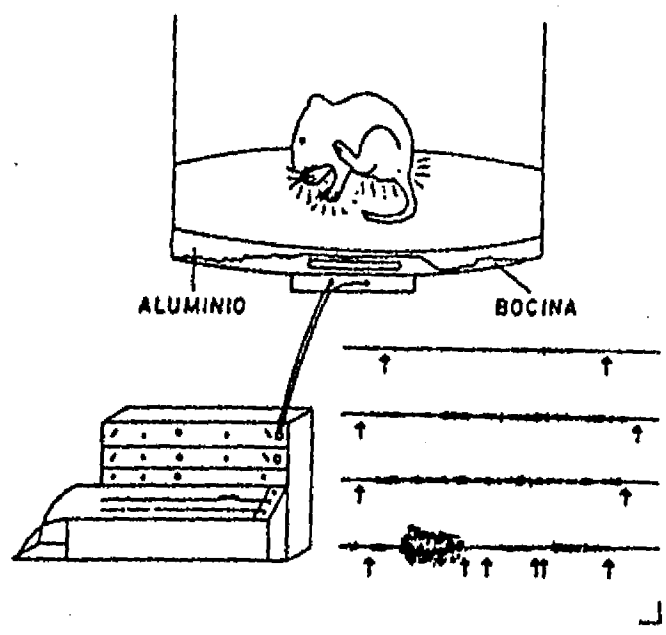
En estudios recientes de nuestro grupo de investigación hemos encontrado que las ratas adultas bien nutridas que fueron desnutridas sólo durante el periodo

neonatal, presentan invariablemente un exceso de autoaseo. Así, cuando a estos animales se les expone en días sucesivos al ambiente novedoso de un campo abierto o en las madres neonatalmente denutridas durante el periodo postparto, incrementan tanto la frecuencia como la duración del autoaseo (Salas y Cintra, 1979; Salas y col., 1984). En otros estudios hemos confirmado que el incremento del autoaseo que se ve en las ratas neonatalmente desnutridas, se da a expensas del lavado de la cara, lavado de la cabeza, lamido de la piel y del rascado del cuerpo con los miembros posteriores (Salas y col., 1991), mientras que el lamido de los genitales y el de los dedos de los miembros anteriores resulta poco modificado (Fig. 8).

Los resultados del último estudio, sugieren que la desnutrición neonatal posiblemente interfiere con la maduración de los circuitos neurales involucrados en la regulación del autoaseo tales como la substancia gris periacueductal (Berridge y Fentress, 1987), el neoestriado, el núcleo acumbens y la vía nigro-colículo-substancia gris central que participan en la modulación del autoaseo (Gispen y col., 1988; Spruijt y col., 1986). Sin embargo, se requerirá en el futuro de más estudios de tipo morfológico de las poblaciones neuronales de estos relevos sinápticos, que den mayor sustento a esta hipótesis.

En el presente se ha hecho evidente el tremendo avanze que ha tenido el estudio de la ontogenia del SNC y de los mecanismos a través de los cuales los factores epigenéticos, interfieren con la organización citoarquitectónica del cerebro y su función. Asimismo, de que manera los trastornos en la organización del substrato neuronal, son relevantes para alterar las funciones plásticas del cerebro y las alteraciones a corto y a largo plazo en la conducta. Sin embargo, a pesar de lo mucho que se ha conocido acerca de los factores externos que regulan el crecimiento del cerebro, desconocemos casi por completo todo el conjunto de factores endógenos que están asociados a la ontogenia neural. Los años venideros serán ricos en este tipo de información y hasta entonces, tendremos la posibilidad de

prevenir y revertir los efectos de agentes nocivos perinatales, que deterioran el substrato neuronal y la potencialidad de sus funciones.



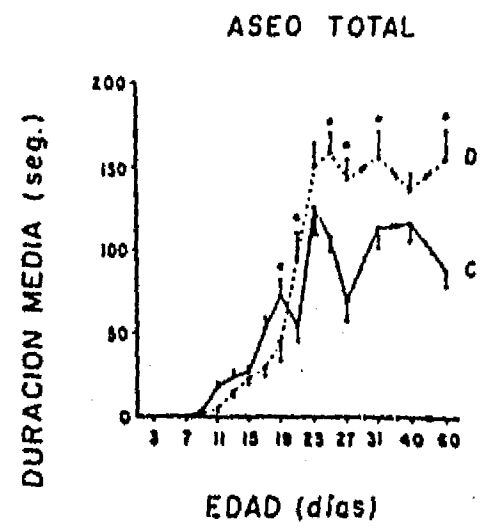


Fig. 8. Registro de los movimientos ocurridos durante la conducta de autoaseo (espacio entre las flechas), y duración media del mismo en ratas control (C) y desnutridas (D) durante el periodo neonatal. Nótese que después del día 23 de edad en el grupo desnutrido, los valores del autoaseo son significativamente mayores cuando se les compara con los del grupo testigo. Modificado de M. Salas y col., Physiol. Behav., 50: 567-572, 1991.

CONCLUSIONES

El desarrollo y la organización citoarquitectónica del SNC, se encuentran determinados tanto por la información genética programada, como por la influencia de factores epigenéticos medioambientales.

El cerebro de la mayoría de los mamíferos pasa durante su desarrollo por una etapa de alta vulnerabilidad (periodo crítico), dependiente de la concurrencia temporal de diversos procesos citogenéticos con la influencia de factores medioambientales, que establecen ciertos límites de sus propiedades plásticas de funcionamiento.

La privación neonatal de alimento, interfiere más con el desarrollo neuronal de estructuras cerebrales conectadas con la esfera sensorial, que con las de la motora. Estas alteraciones están principalmente localizadas a nivel de los árboles dendríticos (órdenes dendríticos, espinas sinápticas y amplitud de los campos dendríticos) y por lo tanto se ven interferidas, la integración y la elaboración de las descargas neuronales.

La desnutrición neonatal, retarda la aparición y el desarrollo de la actividad eléctrica cerebral espontánea y de la provocada por la aplicación de estímulos sensoriales. Asimismo, a través del análisis de ellas, es posible detectar que son los circuitos polisinápticos los que se afectan en mayor grado, comparados con los oligosinápticos.

Las alteraciones morfológicas y electrofisiológicas provocadas por la desnutrición perinatal, guardan una estrecha correlación con el retardo y el desarrollo

de la conducta refleja y con las alteraciones a largo plazo en procesos fisiológicos complejos como la conducta social, la exploratoria, el aprendizaje, la memoria, la respuesta emocional y el autoaseo entre otras.

Algunas de las alteraciones morfológicas y funcionales asociadas a la desnutrición neonatal y a otras influencias nocivas perinatales, pueden ser atenuadas o revertidas a través de la rehabilitación nutricional o de la exposición a distintas rutinas de estimulación sensorial.

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Effects of Early Malnutrition on the Development of Swimming Ability in the Rat[1]

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SALAS, M. *Effects of early malnutrition on the development of swimming ability in the rat.* PHYSIOL. BEHAV. 8 (1) 119-122, 1972.–The ontogeny of swimming ability was studied in normally fed and in chronically starved rats to assess the effect of early malnutrition on CNS development. Swimming tests were performed on adult and infant rats from 4-30 days old in an aquarium. Scoring according to position of the nose and movement of front legs. Maturation of swimming performance was delayed 2-3 days in the chronically starved rats as compared to controls. The high sensitivity of nervous tissue during critical periods of maturation and the relationship between malnutrition and the development of some patterns of movement during swimming are considered.

Early malnutrition Neurophysiological development Neonatal behavior

IT is generally accepted that the events in the internal and external environments are continuously providing a stream of information that pours through the sense organs into the CNS. Thus, evidence indicating the fundamental role of hormones or other chemical compounds during early stages of neuronal development, has been reported [3, 15, 16]. It is also known that external influences like handling and nutrition during early life are also basic factors controlling the maturation of the CNS and which will determine its future functional pluripotential flexibility [1, 4, 6, 17].

Closer packing and a decrease in size of cortical neurones [1, 12], retarded myelination [5], abnormal configuration of the EEG and evoked potentials [9, 14], as well as, behavioral and biochemical changes [2, 7] in the course of acute or chronic undernutrition were previously reported. From these results it was shown that these deleterious effects appear to depend on the timing of the malnutrition in relation to the period of development of the neural substrate.

There are few systematic studies dealing with the normal sequence of swimming development in the rat and mouse [10, 13, 16]. Recently, it was shown that swimming ability and its maturation is a suitable biological tool to assess CNS development and to analyze the integration of the neuromuscular mechanism generally studied in isolation from a completely developed neuromuscular system [16]. In view of the close relationship between nutrition and CNS maturation, the aim of this report is to investigate the role of starvation on the ontogeny of this complex pattern of behavior.

METHOD

Experiments were carried out in 64 infant (1-30 days of age) and 8 adult Wistar rats of both sexes, bred in our laboratory and reared under standard conditions of management and nutrition. Each litter was reduced to eight rats, four were used as control and four as experimental. Chronic starvation was achieved by maintaining the infant rats separated from the mother and litter-mates in an incubator at 29°C for a period of 12 hr daily from 4-13 days of postnatal life (the Day 4 was taken as the first day of the starvation period). After this starvation period, the rats were returned to their cages with the mother and litter-mates.

Daily swimming tests were performed on rats ranging in age from 4-30 days and in adults (120 days). These tests consisted of dropping each rat into the water, which was 20 cm deep and held at 27°C, of an aquarium 25 by 50 by 30 cm. The animals were left in the water for 5-10 sec. The procedure to assess the swimming ability by measuring the nose position and movement of the front legs has been previously described [16]. Photographic records of most swimming tests were also taken. The swimming ability score was graphed as the average of 32 individuals for each

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developmental age. Statistical analysis was done by using the Mann-Whitney U test. Body weight was taken daily and the results were evaluated.

RESULTS

Before 4 days of age both experimental and control animals were unable to swim. They usually displayed uncoordinated movements, floated motionless, partially or entirely submerged, arched their backs, turned their bodies and showed hyperextensive reflex activity in the extremities, toes and tail. When the animal's head was maintained under the water, flexor-extensor head movements were occasionally observed. At this age there was no difference between control and undernourished animals in displaying this poorly coordinated activity. In general, most of the animals remained floating motionless (Fig. 1), during this period of maturation.

From 4-13 days of age both control and undernourished animals tended to improve their swimming ability. At the beginning of this period (Days 4-10), the control animals were able to maintain equilibrium although they still kept the nose under the water. The poor coordination of reflex movements hindering the propelling straight line movements of the animals. However, at this time, the rats generally swam in circles showing occasionally random directionless movements. Swimming circling movements appeared from 4-12 days of postnatal life, and they were overlapped with the end of the floating activity (Fig. 1). From 11 days of age onward the rats maintained the nose, face and a part of the head out of the water as adults (Fig. 2). At this same developmental age they started to swim in straight line, although this motor activity was still mixed with circling movements (Fig. 1). After 12 days of age the straight line movements were consistent as in the adult rats.

A similar sequence of maturation was observed in the starved rats, except that it was delayed by 2-3 days as compared to controls. The starved rats were unable to maintain their noses out of the water until about 13 days of age (Fig. 2). Most of the differences observed in the nose position scores between this group and controls at different ages were statistically significant ($p < 0.05$). The floating movements were similar in both experimental groups. The disappearance of circling movements and the appearance of straight line swimming activity were delayed two days in the starved animals (Fig. 1). In general, the swimming tests indicated that controls tended to behave more actively than the undernourished.

Figure 3 summarizes the changing characteristics of front leg movements during the development of swimming ability of both control and experimental animals. From 11-13 days old in the normally developed rats, the front leg activity consisted of active and well coordinated flexor-extensor movements, but from this time onward the movements progressively decreased until they ceased at about 21 days of age. In the undernourished group a two day retardation in the development of this pattern of motor activity was observed. Figure 3 also shows that in most of the cases, the score differences between undernourished and controls were statistically significant ($p < 0.05$). This period of progressive cessation of foreleg activity lasted 8 days in controls (from Days 13-21) and 10 days in underfed rats (from Days 13-23).

The control rats were significantly heavier and larger than the undernourished after 6 days of postnatal life. A

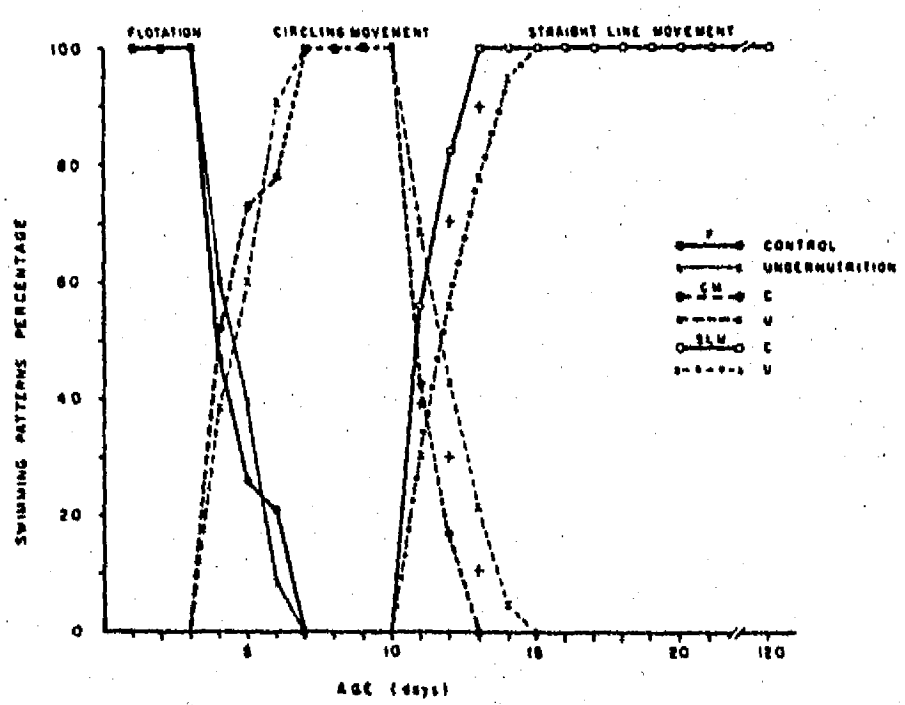

FIG. 1. Results of changing characteristics of motor activity during development of normal and chronically malnourished rats. Points differing from controls at a level of significance of $p < 0.05$ are marked with a cross.

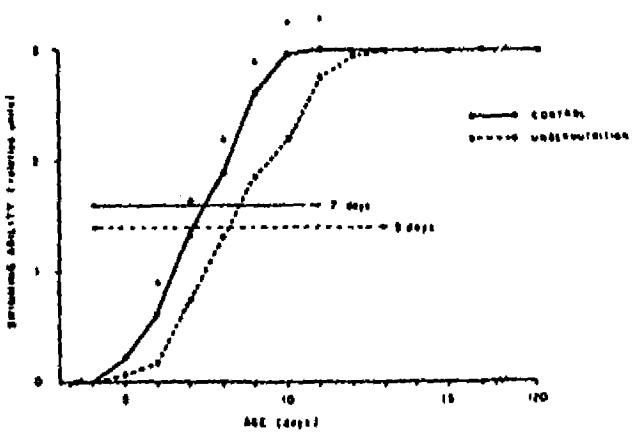

FIG. 2. Results of swimming maturation in normal and malnourished treated rats at different ages. Each point in the graphs represents the average of 32 values. Horizontal lines represent the number of days over which swimming behavior assumed stable characteristics. Points differing from controls at a level of significance of $p < 0.05$ are marked with a cross.

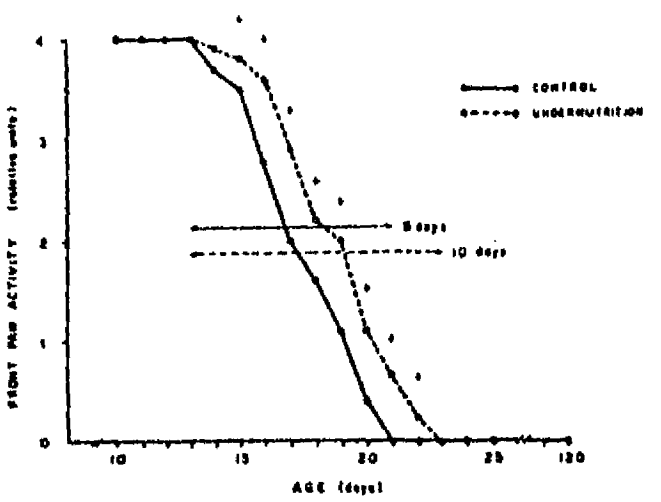

FIG. 3. Changes in front paw activity during development of normal and chronically malnourished rats. Horizontal lines represent the number of days from the beginning to the end of the progressive inhibition of front paw activity. Points differing from controls at a level of significance of $p < 0.05$ are marked with a cross.

mean difference of about 10 g between the two groups all over the experimental period was observed.

DISCUSSION

It is established that malnutrition produces a slower CNS maturation and remarkable deficits in intellectual capacity at a later age [1, 4]. The present report gives evidence that chronic undernutrition affects the maturation of the neural substrate controlling the complex neuromuscular adaptive mechanisms involved in swimming. Thus, rats which have been starved for a period of 10 days in early life showed a significant delay in the maturation of the ability to keep the nose and the head out of the water, in the progressive cessation of foreleg activity and in the development of motor patterns during swimming as compared to controls.

During normal development, it is clear that an adequate supply of different nutritional factors is essential for the multiplication, differentiation and organization of the neural substrate [1, 7]. The neural structures controlling the neuromuscular mechanisms of swimming are unclear. Swimming appears to be purely reflex in the lower mammals [8]. In the rat this activity is presumably determined by the somatosensory cortex, labyrinthine structures and cerebellum. Thus, in adult animals, it is known that these areas maintain wide connections with each other to coordinate space orientation, posture, voluntary movements and equilibrium [8]. Furthermore, the vestibular nuclei have excitatory and inhibitory axons projecting to the cervical and lumbosacral motoneurones that determine the position of the head and limbs [11, 18]. Unfortunately, the morphological development of the vestibular mechanisms to coordinate the position of the head and the foreleg activity during swimming are unknown. In the present results, the undernutrition delayed by 3 days the ability of rats to keep the head and the nose out of the water as well as, to progressively cease the foreleg activity; this delay could be interpreted as a consequence of the morphophysiological disorganization produced by malnutrition on both cortical and brain stem vestibular mechanisms. In fact, a recent study employing histological and microchemical techniques has demonstrated that the somatosensory cortex of the rat is severely affected by undernutrition during its phase of rapid growth [1], and preliminary results in this laboratory indicate that bilateral lesions to the somatosensory cortex in the adult rat severely affect the ability to swim.

In the present results both control and experimental animals exhibited during development three successive patterns of motor activity; flotation, circling and straight line movements. This sequential course of development may also have a relationship with the progressive morphophysiological development of cortical and brain stem mechanisms. Undernutrition does not change this sequential course, but produces a delay in its rate of development.

In conclusion these data provide additional information that early malnutrition affects the neuronal development of the mechanisms involved in swimming. Furthermore, reinforce the general agreement that undernutrition is one of the external environmental factors which, interacting with the growing neuron in the early postnatal life affects the rate of the CNS development on which the later behavioral repertoire is based. In addition, since it is possible in young animals to observe well defined patterns of movement, swimming and its development may be a suitable model to use in studying functional integration of reflex activity.

ACKNOWLEDGEMENT

I am greatly indebted to Leon M. Cintra for his technical assistance in the major part of this work.

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Nutritional Influences Upon Somatosensory Evoked Responses During Development in the Rat[1]

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SALAS, M. AND L. CINTRA. *Nutritional influences upon somatosensory evoked responses during development in the rat.* PHYSIOL. BEHAV. 10(6) 1019–1022, 1973.–Taking the evoked responses as indicators of CNS maturation, the effects of neonatal starvation on cortical evoked activity were studied in rats at different ages during development. Early malnutrition delayed the electrophysiological development of both primary and secondary responses, with the effects upon the secondary slow potentials being more severe than on the primary responses. These data suggest a differential effect of malnutrition upon the projecting system to the neocortex. The possible connection of these results with impaired nervous and mental processes following early malnutrition are discussed.

Neonatal malnutrition Electrical activity development Afferent projecting system

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IT IS well established that undernutrition in the early postnatal life plays a fundamental role in the developing nervous system. Recent studies on the effects of malnutrition upon brain growth have indicated that the somatosensory cortex of the rat is severely affected [3,6]. Thus, persistent and progressive histologic and biochemical abnormalities despite subsequent recovery of body weight are generally seen after a perinatal chronic starvation period [3]. Recently, it has also demonstrated that severely undernourished animals showed a delay in the appearance and development of different behavioral locomotor patterns [1, 15]. However, it appears that most of these locomotor impairments progressively disappear at later ages with the apparent persistence of behavioral and learning deficits.

It is widely known that in response to afferent stimuli the sensory cortex shows electrical responses consisting of a fast primary potential and a series of rhythmic secondary slow waves. These responses have been used very commonly as indicators of CNS maturation. In the study of the effects of food deprivation upon locomotor and behavioral development we were interested in determining if the action of malnutrition would have a differential effect on the maturation of the specific and nonspecific evoked somatosensory responses in the rat. To investigate this possibility evoked cortical responses to sciatic nerve stimulation were studied in rats that were undernourished at different ages during development.

METHOD

A total of 85 laboratory-bred Wistar rats between 5 and 45 days of age were used throughout experiments. In all cases split litters were used, half receiving the experimental treatment and half serving as controls. The procedure used to undernourished infant rats was described in detail in a previous publication [15]. Briefly, litter size was kept constant from the day of birth at 8 pups per mother. The control pups were freely nursed by mothers. The undernourished animals were isolated from the mother and littermates and kept in conditions simulating those in a rat's nest. They were placed in an incubator and held at a temperature of 29°C for a period of 12 hr daily from Days 4–13 postnatal life.

Recording of evoked responses was done on Days 5, 7, 9, 11, 16, 18 and 45 days of age. In the acute experiment surgical procedures were performed under ether anesthesia. In order to permit direct recording from the somatosensory cortex, the infant animals were immobilized with 0.001 mg IP succinylcholine chloride, while adults received 0.004 mg. Artificial respiration was employed with short bursts of air from a mechanical pump connected to a tracheal cannula. The sciatic nerve was prepared for electrical stimulation in its pelvic trajectory. The operative sites were kept moist at all times with mineral oil and the animal's body temperature was maintained by an enclosed circulating water heating pad.

Portions of the scalp were removed so that recording

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[1] This investigation has been aided by a grant from the Foundation's Fund for Research in Psychiatry.

electrodes could be inserted through the skull and meninges to make direct contact with the surface of the brain. The interelectrode distance ranged from 2–3 mm. The animal's head was then firmly clamped in a specially made head holder, and the pressure points were infiltrated with local anesthesia. The electrodes were held in place by the surrounding bone and remained fixed throughout the experiment. Electrodes were sharpened stainless steel insect pins (size 00), insulated except for 1 mm at the tip. A pair of electrodes was placed in the somatosensory cortex from which evoked potentials were recorded following sciatic nerve stimulation. The electrical stimuli were obtained from a SIU5 stimulus isolation unit connected to a S8 Grass model stimulator and consisted of monophasic square pulses of 0.5 msec duration and 1–2 V intensity, one stimulus was presented every 5 sec.

Evoked potentials were amplified and recorded with standard neurophysiological equipment including type 122 low level Tektronix preamplifier, 502A Tektronix oscilloscope and a model C4K Grass camera. In all the experiments 50–60 single evoked responses as well as, several pictures of 5 and 10 superimposed successive potentials were recorded. The mean peak latency was measured and graphed and the *t*-test applied. To analyze the characteristics of secondary responses, 30 single sweeps were manually superimposed and the percentage of animals showing consistent secondary responses were calculated in each experimental group during development. Before acute experiments the body weight was taken and then the mean value in each age group was calculated and graphed (Table 2).

TABLE 1

PERCENTAGE OF CONTROL AND UNDERNOURISHED ANIMALS AT DIFFERENT AGES (DAYS) EXHIBITING SECONDARY RESPONSES AFTER SCIATIC NERVE ELECTRICAL STIMULATION

Ages (days)	Number of animals		Percentage of secondary responses	
	Control	Undernutrition	Control	Undernutrition
5	5 (6)*	2 (6)	83.3	33.3
7	7 (7)	2 (7)	100	28.5
9	6 (6)	3 (6)	100	50
11	5 (6)	4 (6)	83.3	66.6
16	5 (5)	5 (6)	100	83.3
18	5 (6)	4 (6)	83.3	66.6
45	4 (5)	4 (7)	80	57.1

*Total number of animals

TABLE 2

EFFECTS OF UNDERNUTRITION ON BODY WEIGHT OF RATS AT DIFFERENT AGES

Age (days)	Mean body weight of animals	
	Control	Undernutrition
5	12	9
7	15	11
9	20	14
11	23	16
16	30	20
18	31	22
45	72	53

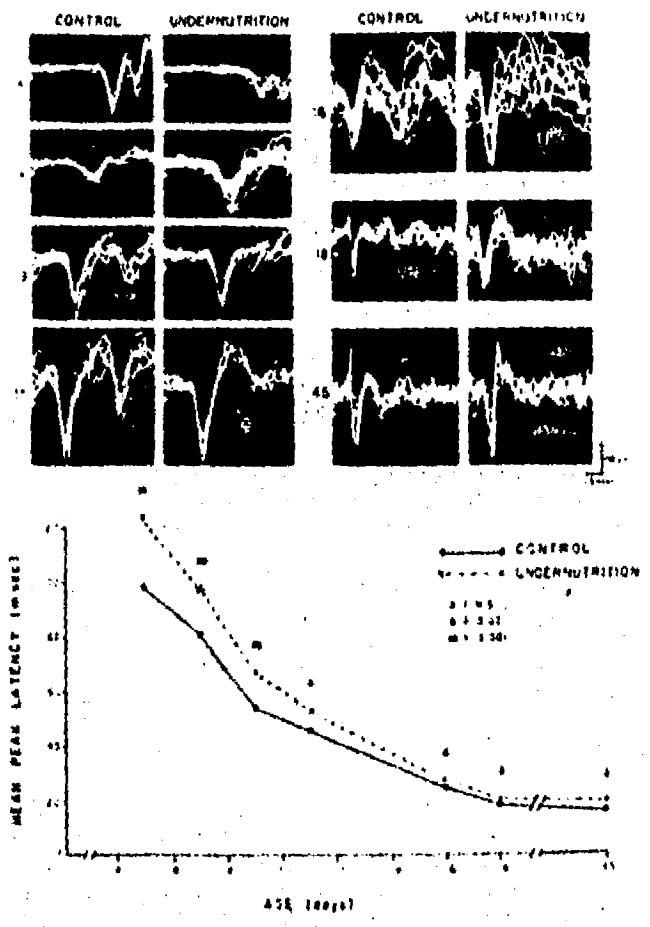

FIG. 1. Development of somatosensory evoked responses in controls and malnourished animals. Control rats show shorter mean peak latencies before 16 days of age than starved rats. Each point in the group represents the average of 250 responses.

RESULTS

Figure 1, illustrates the maturation of somatosensory evoked potentials in normal and undernourished animals. In normally developing rats, contralateral sciatic nerve stimulation elicited responses on the fifth postnatal day, the earliest time period studied. At this age the primary response consisted of a long latency prominent positive component. With increasing age the waveform of this response changed to a biphasic configuration at about 11 days of age. Concerning the secondary response, it was consistently present and also changing in configuration at all ages tested (Figs. 1 and 2). It is interesting to note that at 11 and 16 postnatal days, both primary and secondary components of the evoked response exhibited higher amplitude and variability than at other ages during development (Fig. 1).

In the group of underfed animals at the ages of 5, 7, 9

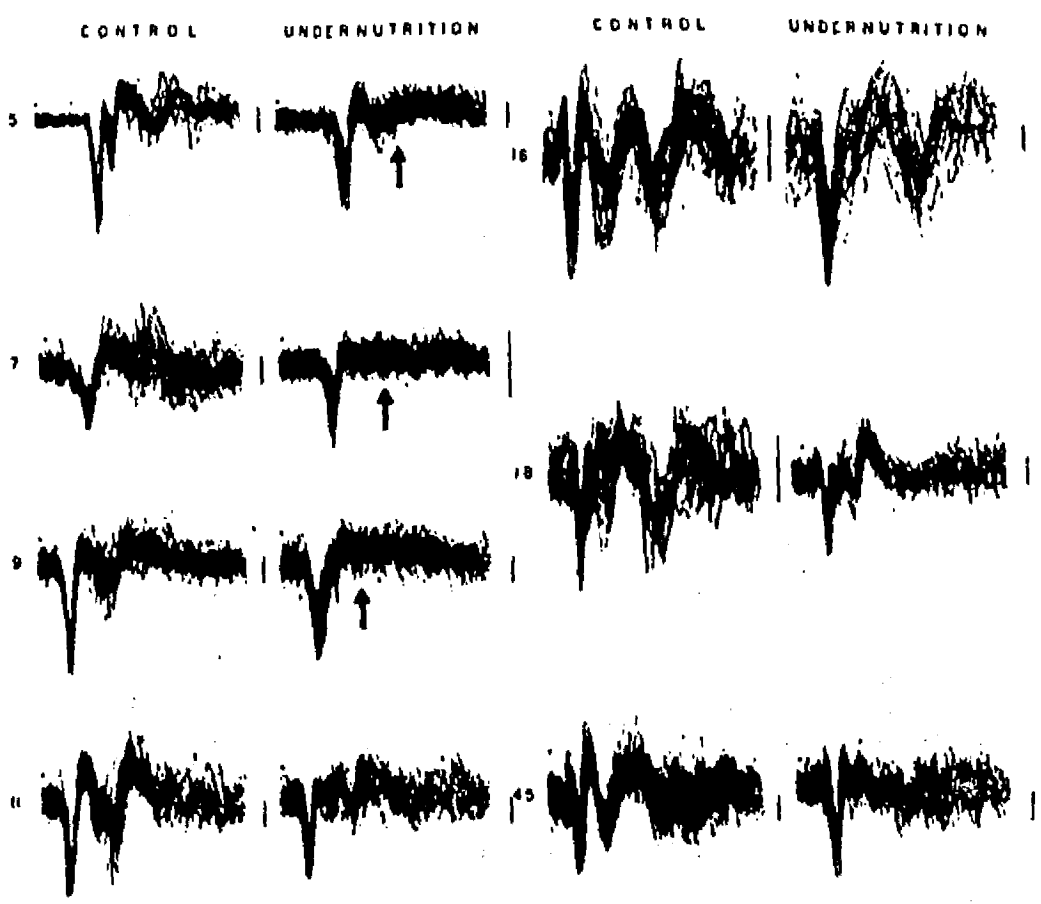

FIG. 2. Projected oscillograms manually taken from records and oriented in time with respect to initial primary response recorded in maximal potential region. Arrows at 5, 7 and 9 days of age indicate the absence of responses; from 11 days onward these responses were consistent but still smaller than controls. In each frame 30 successive responses were superimposed. Calibration: 50 msec and 20 μV.

and 11 days after birth, there was a statistically significant prolongation ($p<0.05$) in the mean peak latency of primary response; however at 16, 18 and 45 days of postnatal life this prolongation in latency was not statistically significant (Fig. 1). At 5, 7 and 9 days of age the secondary response was severely affected in its appearance (Fig. 2). However, from 11 days onward there was a tendency to increase the number of secondary responses. Notwithstanding, this response was smaller in amplitude than in controls. The increment in amplitude and variability of both primary and secondary responses seen in the controls at 11 and 16 postnatal days was also observed in these animals.

Table 1 summarizes the percentage of total number of control and undernourished animals at different developmental ages in which primary evoked responses were followed by secondary responses. In the control group the proportion of secondary responses was higher than in underfed rats.

DISCUSSION

The foregoing data have indicated that there is a definite sequence in the development of somatosensory responses with age in the rat. Thus, control animals exhibited long latency monophasic positive primary responses from the earliest time period studied; there was a progressive reduction in mean peak latency of primary responses with age achieving the adult values at 18 days old; and waveform gradually changed from monophasic to a biphasic configuration (11 days old), attaining adult characteristics at about 16 days old. Furthermore, from 5 days onward there was a progressive tendency for consistent secondary slow waves to appear following the primary responses. In the group of underfed rats the primary responses were monophasic and of longer latencies than those of controls. With increasing age their latencies were progressively reduced, and the waveform changed to a biphasic configuration, as occurred in normally developed rats. Furthermore, the percentage and consistency of secondary responses were smaller than controls.

It is generally accepted that the cortical evoked responses are the result of neuronal processes in response to two different afferent messages to the neocortex [2]. The first message is developed through the specific projection system, and the second message relayed via collateral afferents from the lateral sensory pathways to the multineuronal nonspecific system. The present results indicate that early malnutrition affects the development of both specific and nonspecific thalamocortical projection systems. Concerning the increase in mean peak latency of primary

response and secondary response alterations produced by starvation, these effects might be due not only to a delay in the process of myelination [3,7], number of axons terminals [6], but also to a deficiency of proteins and delayed biochemical maturation of the synaptic transmission in the young animals [3, 6, 12]. These data also agree with previous reports that early malnutrition delays the latency of the first positive component and the waveform of visual and auditory evoked cortical potentials development in the rat [5, 11, 12]. However, our results not only emphasize about the changes in primary repsonses but also in the secondary slow waves, which is widely known play a- fundamental role for nervous activity integration at neocortical structures [2,10].

We would like to emphasize that the effects of early malnutrition upon nonspecific projection system seem to be more impc'tant and presumably more fundamental for the future CNS capabilities, than the effects on the specific system. This observation is functionally very significant since it is well established that the nonspecific system plays a fundamental role in the elaboration of arousal, learning, attention, perception, emotional behavior as well as, in the control of electrical neocortical rhythms [10]. Following this idea it might be possible that the low learning capacity [4], acute wakefulness and sleep effects [8,9] and presumably the emotional disturbances seen in adult rats during starvation may be due in part to disruption of the nonspecific system. In this regard preliminary results in this laboratory indicate that adult rats deprived of food during early infancy, exhibit less variability in the secondary peaks of their evoked responses as well as, showing less exploration and urination when placed in a novel environment. Although, our results are very suggestive that early malnutrition would differentially affect the development of the specific and nonspecific systems, at this time we cannot conclude that the nonspecific system damage is the only or even the most important explanation of the low behavioral capabilities exhibited by rats as a consequence of perinatal malnutrition [4,13].

The classic work of Small [16] indicates that at about 14 days of age, rats exhibit a remarkable locomotor development; they are able to walk, jump and climb over considerable obstacles. These motor skills improve at 15 and 16 days of age when ear and eye-opening occurs. In our results at 11 and 16 days of age a considerable variability of evoked somatosensory responses was observed. This variability might indicate increased excitability of central motor structures as a result of the increment in neuromuscular activity during development. This observation is strenghtened by a previous report [14] suggesting that the stimulation of muscle nerve produces large evoked secondary discharges in the somatosensory area.

In conclusion, the present electrophysiological results have a close relationship with previous morphological, electrophysiological and behavioral changes produced by malnutrition during development.

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EFFECTS OF NEONATAL FOOD DEPRIVATION ON CORTICAL SPINES AND DENDRITIC DEVELOPMENT OF THE RAT

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SUMMARY

The large cortical pyramidal cells of layer V of the frontal and occipital areas have been examined with Golgi staining in normal and malnourished infant rats.

The number of spines, the basilar dendritic density and the dendritic thickness were significantly reduced in the group of starved rats. The functional significance of these findings is discussed in relation to other morphological and electrophysiological changes in cortical structures, and their possible implications upon the physiological integrative processes of the CNS at later ages in life.

INTRODUCTION

It is well documented that malnutrition at critical stages of development may affect the mammalian brain adversely. Thus, inadequate nutrition of newborn rats results in reduction of brain weight[6], delayed myelination[5,7], close packing of cortical neurons and loss of axon terminals in the cortex[6,9]. These morphological impairments are correlated with a deficient behavioral, electrical and biochemical development[3,4,5,14,16–18].

The dendritic spine is a distinctive structure of the postsynaptic apparatus allowing the dendrite to synapse with axon terminals[11,12]. Observations of rats and kittens show that in these species the cortical dendrites lack spines at birth. The development of dendritic spines with increasing age is closely correlated with the development of behavioral and neurophysiological characteristics, and it has therefore been suggested that the growing spines may provide a morphological foundation upon which later physiological capabilities are established[19].

The present study was undertaken to investigate the effects of malnutrition on the development of dendritic spines, and the dendritic prolongations in the large pyramidal cells of cortical layer V of the rat.

METHODS

For this study 4 split litters of both sexes of infant Wistar rats bred in this laboratory were used throughout. In order to standardize the conditions of management and nutrition each litter was reduced at birth to 8 pups/mother. Four rats of each litter were used as controls and 4 as experimentals. The controls were left untouched until they were sacrificed while experimentals were gently removed from their cages to an incubator maintained at 27–28 °C, for a 12-h period of food deprivation. The condition of undernutrition was maintained between day 4 and day 13 of postnatal life.

At 7, 9, 12 and 15 days of age each member of the litter was weighed and killed. After that, a 3-mm wedge of brain through the frontal and occipital cortical areas was removed and placed in a solution of osmic acid and potassium dichromate. Two brains of controls and 3 of malnourished rats were chosen at random for the morphological analysis. The sections were stained by a variant of the rapid Golgi technique[20]. After the cortical wedges were fixed and stained with silver nitrate, 20–30-μm transverse sections of the brain were progressively washed in water and dehydrated in 4 different concentrations of alcohol, and xylene. After this procedure they were mounted under neutral natural resin. Slides were coded and blind counts were tabulated at × 785.5 magnification. A minimum of 10 cortical pyramidal neurons/cortical area were studied on each animal. The number of spines was counted in apical, terminal, oblique and basilar dendrites in a 69-μm extent/dendritic prolongation. The dendritic density was estimated at the level of the basilar dendrites. Counts were made by a sampling method using a grid of 100 small squares placed in the ocular of the microscope so that the image of the section fell in the grid at a × 500 magnification. The cell body was then placed in the center of the grid and from the 30 squares surrounding the cell body the number of fibers falling within 6 squares selected at random were counted. Thus fibers in 60 small squares on a total of 10 neurons were counted and the average number was taken for comparative analysis as an indicator of the dendritic density.

The dendritic thickness in micra was determined by sampling in the different dendritic prolongations with an ocular micrometer. Since the dendritic thickness normally decreases from the origin of the dendrite to the periphery, the counts were performed in the first 25 μm close to the origin of the dendritic branch. From a total of 20 neurons 16 different samples in each cell were selected from the different dendritic prolongations indicated above.

The experimental data were analyzed by the t test to estimate the validity of the differences between means resulting from the experimental treatment.

RESULTS

Both groups of rats showed an increasing number of spines in the frontal and occipital areas with advancing age (Fig. 1). Compared to the controls, however, the malnourished rats showed a lower number of spinal processes ($P < 0.05$). It is

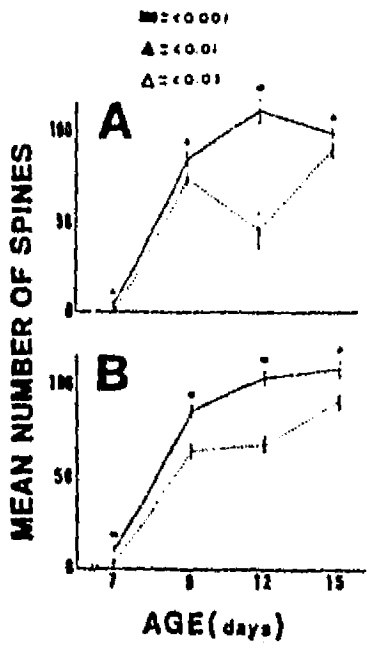
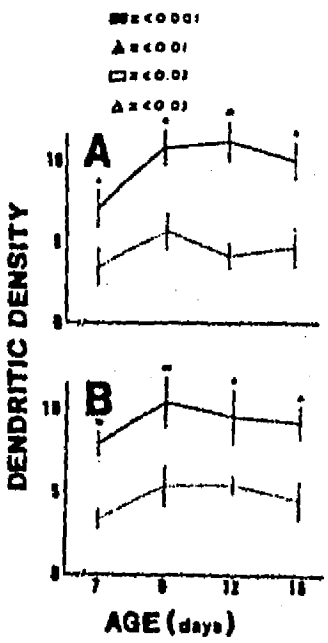


Fig. 1. Effect of early malnutrition upon spine development of the large cortical pyramidal cells. A: frontal cortex. B: occipital cortex. Each point in the curves represents the average of the counts obtained in a minimum of 9 neurons. Solid line, control rats; broken line, malnourished rats.

interesting to note that the reduction in number of spines took place without any change in the distribution of the various forms of spines on the dendritic tree, *e.g.*, the majority of the stubby spines occurred close to the cell body while the thin spines were mostly found in the periphery of the dendrites. In addition, at 12 days of age,



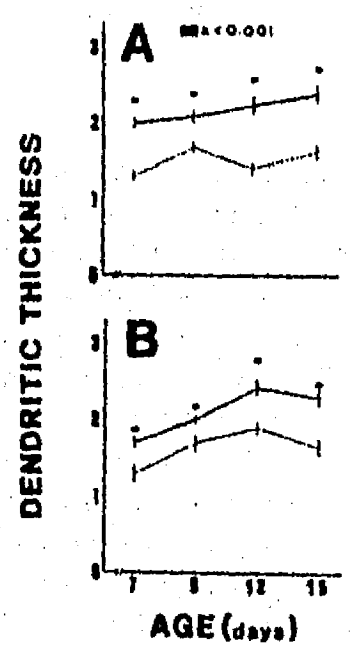


Fig. 2. Effect of neonatal malnutrition upon dendritic density development of large cortical pyramidal cells. A: frontal cortex. B: occipital cortex. Each point in the curves represents the average of 60 samples obtained in a total of 10 neurons. Solid line, control rats; broken line, malnourished rats.
Fig. 3. Effect of early malnutrition upon dendritic thickness of the large cortical pyramidal cells. A: frontal cortex. B: occipital cortex. Each point in the curves represents the average of 160 samples taken in a total of 10 neurons. Solid line, control rats; broken line, malnourished rats.

TABLE I

EFFECTS OF MALNUTRITION ON BODY WEIGHT OF RATS DURING DEVELOPMENT

Age (days)	*Mean body weight (g)*		*Weight decrease in experimental rats (%)*
	Control	*Experimental*	
7	13.8 ± 0.16	11.2 ± 0.28	18.9
9	21.7 ± 0.25	17.0 ± 0.40	21.7
12	23.0 ± 0.40	15.5 ± 0.47	36.1
15	31.2 ± 2.31	21.7 ± 0.86	30.5

the frontal area exhibited a marked reduction in the number of spines compared to controls. A similar tendency was observed in the occipital area at the same age.

Fig. 2 shows the progressive increase with age of the complexity of the basilar dendritic network, and Fig. 3 shows the variations occurring in the thickness of the dendritic prolongations. Comparing the two groups of animals the starved rats showed a reduced complexity of the basilar dendrites ($P < 0.05$) and reduced thickness of the dendritic prolongations ($P < 0.05$). Particularly marked was the reduction taking place in the thickness of the dendritic extensions in the frontal cortex of the starved rats at 12 days of age.

The average body weight of malnourished littermates was 19–36% below that of the normally fed littermates (Table I). Moreover, the size of malnourished rats was reduced at all ages when compared with controls.

DISCUSSION

Early malnutrition was observed to result in reduction of the total number of dendritic spines, a decrease in the density of the basilar dendritic network and a reduction of the thickness of the dendritic prolongations of the large pyramidal cells. These effects might primarily be due to direct lack of dietary nutrients and particularly to a deficiency of proteins which according to several authors could be responsible for the limitation on brain growth[5,9,16]. The morphological deficiencies observed may, however, also be an indirect result of starvation. One possibility is that the delayed development was due to a loss of cortical afferents in the malnourished rats[6]. This assumption is supported by previous morphological observations which suggest that both subcortical and cortical neurons may normally develop dendritic prolongations and form synapses as a result of intercellular contacts with the growing afferent axons[1,3,13]. Another possibility is that the starvation produced a delayed development of the sensory systems which, in turn, reduced the traffic of sensory impulses reaching the large pyramidal cells, thereby decreasing the stimulation necessary for the normal development of dendritic branches and spines. Studies of effects of light deprivation and handling upon the number of cortical dendritic spines in mice and rats, give some support to this assumption[10,19,21]. A third possibility is that neonatal malnutrition represents a stressor agent that stimulates glial

cell proliferation, which may interfere mechanically with the growth and distribution of pyramidal dendritic branches and with spine proliferation. This possibility is supported by studies showing that various stress conditions such as dehydration, increased sensory and motor activity in both adult and infant result in an increase in the rate of glial multiplication at different levels of the brain[8,12,15]. At the present time no choice among these various possibilities can be made.

Finally, we would like to emphasize the physiological significance of the present data. It has been suggested that basic CNS patterns of interconnection among neurons in early life provide a neuroanatomical basis upon which more complex physiological phenomena are based at later ages[19]. Furthermore, it is generally accepted that the CNS integrative processes in response to afferent stimulation, occur within areas of widely interconnected neurons such as the neocortical structures. Our results indicate a significant reduction in the number of spines, and in the thickness and density of the dendritic tree, which may diminish both the capacity for new interconnections, and the possibility for potential neuronal interactions. The reduced complexity of neocortical structures might be partly responsible for the diminished learning capacities and the reduced adaptive behavioral patterns generally seen in adult mammals deprived of food during early life[4].

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Development of the Electrocorticogram During Starvation in the Rat[1]

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SALAS, M. AND L. CINTRA. *Development of the electrocorticogram during starvation in the rat.* PHYSIOL. BEHAV. 14(5) 589–593, 1975. – The effect of early malnutrition on the electrocorticogram (ECoG) of the rat was studied at eight different developmental stages. Gross observations of the ECoG records indicated that the normal ECoG activity developed from the fifth day; its characteristics consisted of low amplitude slow waves in the 1–15 cps range with a dominant frequency ranging from 6 to 8 cps. From the 7th to 11th postnatal days the proportion of fast activity in the 10–20 cps range progressively increased. This increment was associated with an augmentation in amplitude. The adult ECoG characteristics were achieved at the 18th postnatal day. In malnourished rats, a similar sequence of development was seen; compared to the controls, however, the malnourished rats showed an increment in the proportion of slow waves and a reduced voltage of ECoG up to 16 days when the differences disappeared. An average frequency analysis of the taped ECoG data indicated that in normally fed rats there was a rapid increment in the range and mode of frequency distribution from 5 to 16 days of age. From 18 to 30 days this increment followed a slowly ascending course, and by 30 days the averaged activity was similar to that of adults. Frequency distribution histograms of starved rats followed a similar sequence of development except that at all developmental ages (5–180 days) they exhibited slower frequencies.

Electrocortical development Neonatal malnutrition

DIFFERENT investigations on growth of the brain of the rat have established that food deprivation during the first three weeks of life results in long-term cerebral lesions [5, 8, 9, 10, 22]. These morphological abnormalities have been correlated with marked alterations in the behavioral, biochemical and electrical characteristics of the nervous tissue [1, 5, 7, 13, 20, 21].

Reports of EEG disturbances under severe malnutrition have been reported, mostly in humans [12, 15, 16]. The cortex was observed to exhibit a decreased responsiveness to photic stimulation, and the EEG activity was greatly diminished in voltage and frequency. It was suggested that severe undernutrition in childhood provokes an impeding effect on the normal development of brain electrical rhythms, and that recovery from starvation is paralleled by a partial removal of this influence. To our knowledge, no experimental work on animals has been performed on effects of neonatal undernutrition upon the development of the brain electrical activity.

The present study was undertaken to investigate the effects of neonatal undernutrition upon the electrical activity of the developing rat brain as measured by the ECoG.

METHOD

Animals

Acute electrophysiological observations were made of 96 male Wistar rats bred in this laboratory. At each developmental age 6 animals constituted the experimental group and 6 the control. The procedure employed in producing undernourished rats has been described elsewhere [20]. Briefly, litters were equalized to 8 at birth, 4 rats being used as controls and 4 as experimentals. The control pups were freely nursed by the mother whereas the malnourished rats were isolated from the mother as well as from their littermates and kept under conditions simulating those of a rat's nest. They were placed in an incubator and held at a temperature of 27–28°C for a period of 12 hr daily from Days 4–13 postnatal life. After the starvation period was ended rats were maintained on ad lib water and Purina food pellets. Body weights are showed in Table 1.

Procedure

All surgical procedures were performed under ether anesthesia. The animals were allowed to recover from the anes-

[1] This investigation has been aided by a grant from the Foundation's Fund for Research in Psychiatry.

TABLE 1

EFFECTS OF FOOD DEPRIVATION ON BODY WEIGHT OF RATS DURING DEVELOPMENT

Age (Days)	Mean (± S.E.) Body Weight (g) Control	Starved	% Weight Decrease In Experimental Rats
5	9.68 ± 0.47	7.66 ± 0.28	20.82
7	13.20 ± 0.70	10.11 ± 0.98	23.35
9	17.60 ± 0.37	13.61 ± 0.47	22.63
11	19.11 ± 0.81	12.75 ± 1.60	33.30
16	23.30 ± 1.48	17.61 ± 1.47	24.39
18	34.61 ± 3.05	24.45 ± 1.93	29.37
30	70.43 ± 5.03	56.55 ± 10.43	19.71
180	305.31 ± 16.76	290.58 ± 14.72	4.82

thesia before starting the experiments. Succinylcholine chloride (0.001–0.004 mg/Kg, IP) was used to immobilize the animals in order to permit direct recording of electrical activity from the specific neocortical areas. Artificial respiration was administered with short bursts of air from a mechanical pump connected to a tracheal cannula. Respiration rhythm was adjusted to approximately 50/min and pulmonary excursions were maintained within physiological ranges as determined by direct observations. The good condition of the preparations were based on the assessment of the ECoG, the cortical responsiveness of the animal to auditory and visual stimulation, body temperature and the animal's skin color. The operative sites were kept moist at all times with mineral oil and the animal's body temperature was kept constant by an enclosed circulating water heating pad. The ECoG was recorded from 5, 7, 9, 11, 16, 18, 30 and 180 days of age. The technical procedure followed in order to hold the animal's head and to amplify and record the ECoG from the frontal (FR), temporal (TR) and occipital (OR) regions was similar to that previously described [21]. The scalp, incision sites and the pressure points were infiltrated with local anesthesia (1% xylocaine).

The ECoG was amplified and recorded on a Grass 79C EEG polygraph and stored on a SP-300 FM Ampex tape recorder when the preparations were in an apparent alert or semialert state. The time constant used for these experiments was 0.02 sec coupled with a low frequency filter of 8 cycles. In all experiments the ECoG was also monitored on an oscilloscope (Tektronix 502-A). The taped data of 6 control and 6 experimental animals in each developmental age were subjected to an average frequency analysis by using a 5321-B electronic counter (Hewlett-Packard). In all records the counter was set to analyze at 1V of input sensitivity, 50 successive samples of 1 sec duration at intervals of 4 sec. From these data, frequency distribution histograms in both normally fed or malnourished rats were obtained. The difference between the total amount of samples taken in 6 control vs 6 malnourished rats at each developmental age was also statistically analyzed by the t test.

RESULTS

Gross observations of records indicated that neither in controls nor in malnourished rats could consistent electrocortical activity be detected before 5 days of age. Most of the activity consisted of prolongued periods of silence with very low amplitude slow waves. Nevertheless, from 5 days onward, consistent electrical activity was recorded from each of the cortical regions studied. At this age, the ECoG of normally fed rats exhibited low amplitude, slow waves in the 1–15 cps range with a dominant frequency ranging from 6 to 8 cps. From the 7th to 11th postnatal days the proportion of fast activity in the 10–20 cps range progressively increased. This increment in frequency was associated with an augmentation in amplitude. After 18 days of age the ECoG has assumed adult appearance. In the group of malnourished rats a similar sequence of development was observed. Compared to the controls, however, the malnourished rats showed an increment in the proportion of slow waves and a reduced voltage up to 16 days when the differences disappeared.

Analysis of the taped data confirmed the above findings as far the general features of the development alterations are concerned. However, a more detailed quantitative analysis revealed group differences in the behavior of the control and the malnourished animals which to some extent still remained in adult age. The main results of this analysis are presented in Figs. 1, 2 and 3. Between the 5th and 16th day the control rats exhibited a significant proportion of faster frequencies than the starved animals ($p<0.001$). In this age range, the starved rats showed a dominance of slow frequencies. With increasing age, however, both groups, showed a progressive increment of fast frequencies. Between 16–30 days of age, the rate of increment was slowed down, although a considerable overlapping persisted during this period ($p<0.001$). In adult animals the controls still showed a significant proportion of faster frequencies ($p<0.001$). However in the temporal region the deviations in the electrical activity where higher than in the frontal and occipital regions (FR, $t = 5.64$, $p<0.001$; OR, $t = 3.57$, $p<0.001$; TR, $t = 9.62$, $p<0.001$).

DISCUSSION

The data presented in this paper are in general agreement with previous reports on humans [12, 15, 16] and animals

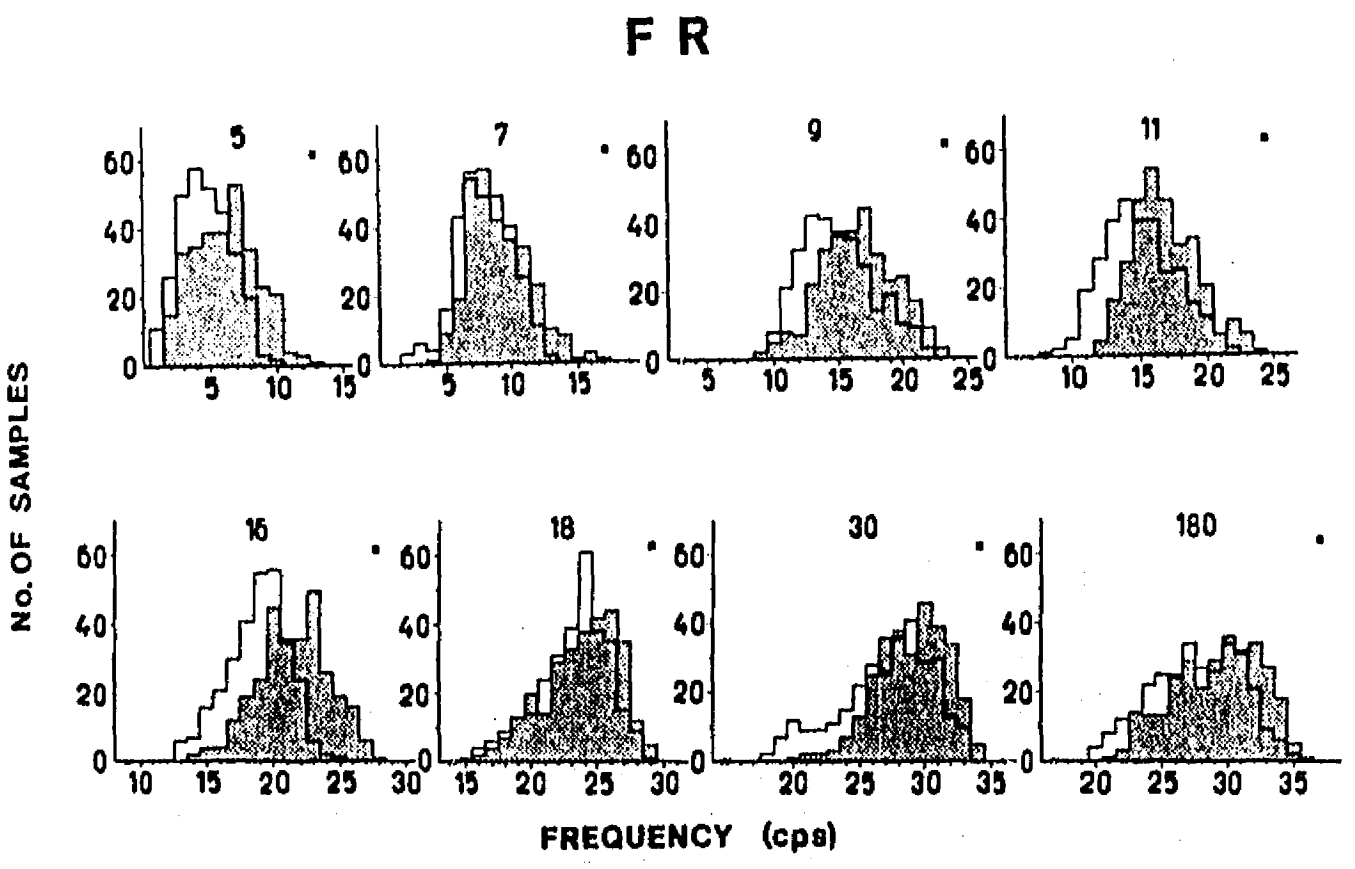

FIG. 1. Average frequency distribution histograms obtained from the frontal cortical region (FR) during development. There is a fast increment in the mode and range of histograms from 5 to 16 days of age, and thereafter this effect is progressively attenuated until 30 days when adult characteristics are apparently attained. Shaded histograms, control rats; white histograms, malnourished rats. Starved rats histograms differing from controls at a level of significance of $p<0.001$ are marked with a black square. Each histogram represents the summation of 300 samples.

[14,18] that early malnutrition may exert impeding effects on the development of spontaneous cortical rhythms. Gross observations of the ECoG of malnourished rats showed the occurrence of a marked increase of the proportion of slow waves and a reduction of the voltage before 16 days of age. By 18 days the adult characteristics seem to have been attained. Average frequency analysis of the ECoG in starved rats indicated that at all developmental ages (5–30 days) there was a marked increment in the proportion of slow frequencies compared to controls. These effects were clearly observed from 5 to 16 days of age. They were thereafter gradually attenuated until 30 days of age. In adult starved rats statistically significant differences in the average frequency analysis still were obtained, exhibiting the temporal cortical region a higher proportion of slow frequencies than frontal and occipital cortical areas.

According to previous observations, malnutrition of newborn rats results in retarded myelination [9], reduction of brain weight [8], delayed cortical lamination [5], close packing of cortical neurons [10], loss of axon terminals in the cortex [8], reduction in number of spines, basilar dendritic density and dendritic thickness [22], retarded caliber growth and irreversible impairment in the perineurial diffusion barrier of peripheral nerves [23,24]. Maturational changes produced by malnutrition may result in profound alterations in the cell interactions and in the membrane properties of excitable cortical neurons and this, in turn may produce corresponding changes in the ECoG of the rat.

Morphological and electrophysiological studies [2, 3, 6] have established the specificity of axonal connections to a restricted part of the neuron. Thus in the cortical pyramidal cells the excitatory synapses are mainly restricted to the dendritic spines, whereas inhibitory connections occur on dendritic bases and on the cell body [11,19]. In a recent publication we have reported that in rats deprived of food early in life there is a marked reduction in total number of pyramidal cortical spines [22]. These findings may partly explain the predominance of slow frequencies in the starved rats. Presumably a considerable decrement both of excitatory connections and inhibitory synapses occurred in our animals.

Finally, it is of interest to mention that, although gross observations on the ECoG of both groups of rats exhibited no differences after 18 days of age, the frequency analysis indicated that the effects of starvation were still present even at 180 days of age. The persistence of a higher proportion of slow frequencies in the temporal ECoG of adult starved rats, supports the view that the temporal lobe is particularly sensitive in the early postnatal period to the noxious physiological disturbances provoked by food deprivation [12,25].

Further support of this suggestion comes from studies of

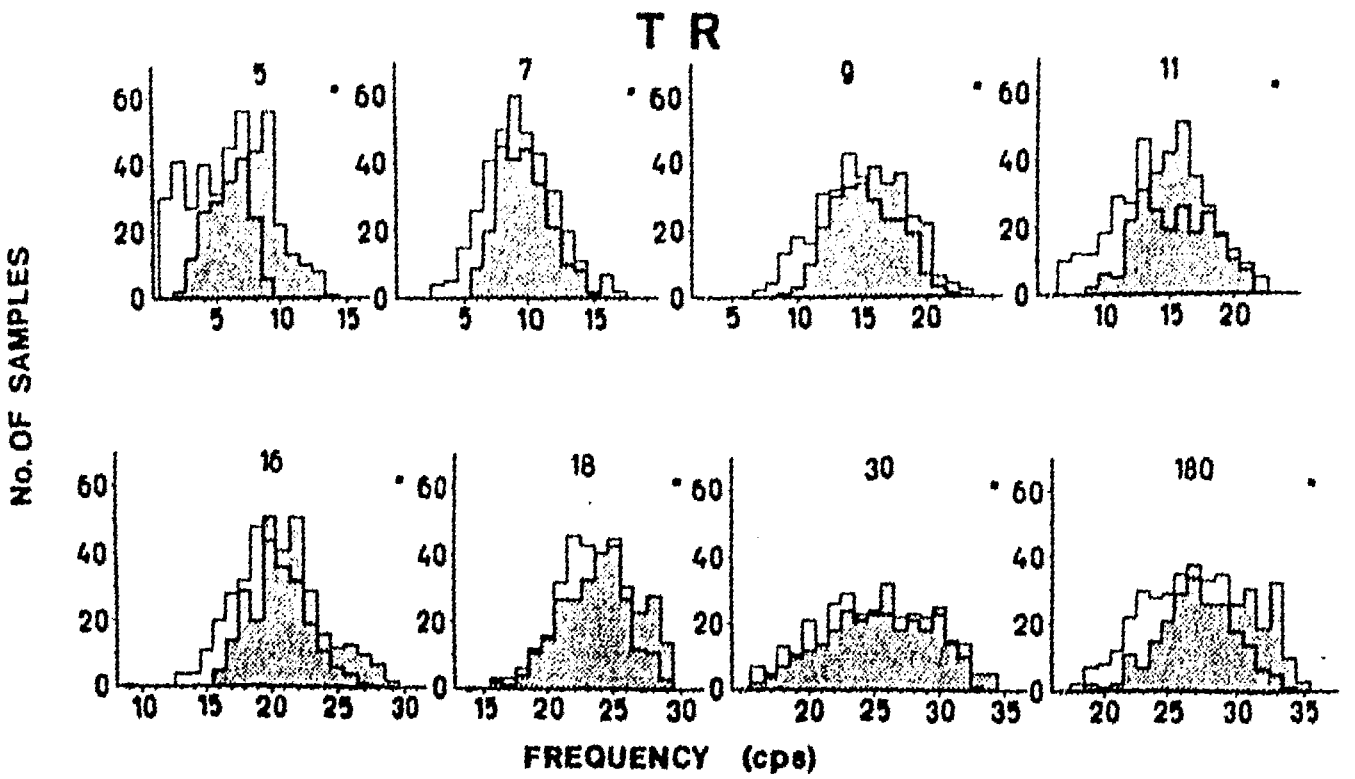

FIG. 2. Average frequency distribution histograms recorded from the temporal cortical region (TR) during development. Note the fast increment in the mode and range of histograms from 5 to 16 days of age, and the slow ulterior effect until 30 days when adult characteristics are apparently achieved. Shaded histograms, control rats; white histograms, malnourished rats. Starved rats histograms differing from control at a level of significance of $p<0.001$ are marked with a black square. Each histogram represents the summation of 300 samples.

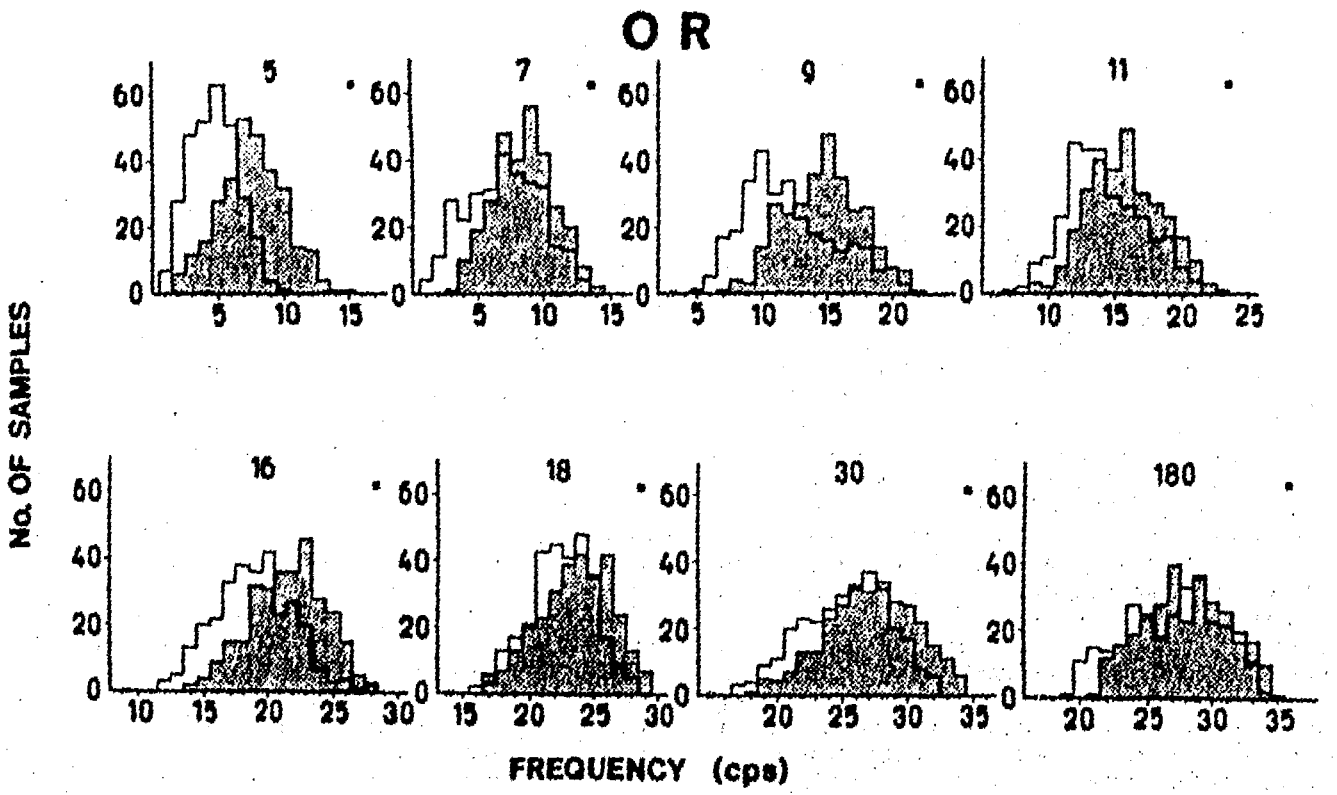

FIG. 3. Average frequency distribution histograms obtained from the occipital cortical region (OR) during development. There is a fast increment in the mode and range of histograms from 5 to 16 postnatal days, and thereafter the effect is progressively reduced until 30 days when adult characteristics are apparently achieved. Shaded histograms, control rats; white histograms, malnourished rats. Starved rats histograms differing from controls at a level of significance of $p<0.001$ are marked with a black square. Each histogram represents the summation of 300 samples.

mentally retarded children [4,17] and from our own observations of undernourished rats showing that the late components of evoked responses have a larger amplitude presumably because of a defect in the maturation of the CNS inhibitory mechanism.

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Long-Term Alterations in the Maternal Behavior of Neonatally Undernourished Rats

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SALAS, M., C. TORRERO AND S. PULIDO. *Long-term alterations in the maternal behavior of neonatally undernourished rats.* PHYSIOL BEHAV 33(2)273–278, 1984.—The effect of early food and sensory deprivation on the maternal responsiveness of female rats was investigated. Animals that were neonatally undernourished by daily mother-litter separation (involving both food and sensory deprivation) showed significant deficits in maternal care, consisting of a reduction in nest rating, nursing time, and retrieving responses. Moreover, they exhibited exaggerated grooming and circling movements in comparison with the controls. Dams neonatally undernourished by the nipple-ligation of their mothers (a method that minimizes sensory deprivation) displayed less alterations in maternal behavior, and no significant differences in grooming and circling from the controls. The data suggest that nest rating, nursing time, and retrieving latency are closely related to food restriction, while the frequency of grooming and circling behavior are primarily associated with sensory deprivation. These results support the view that environmental influences related to food intake and sensory stimulation, interacting at critical stages of brain development, are essential for the maturation of adult behavioral patterns.

Neonatal undernutrition Sensory deprivation Maternal response Rats

SEVERAL studies have suggested that undernutrition throughout gestation and/or suckling has lasting effects on the social behavior [8,33], responsiveness to novelty [13] and aggressiveness [8,13] of the progeny. A variety of methods of food deprivation have been used in these experiments such as reducing the time pups spend with their mothers, increasing the litter size, and restricting the food intake of the dams [20,34]. Although the behavioral alterations noted in these studies are associated with the nutritional state of the mother and/or the young, there are disruptive environmental influences present as well, such as impaired mother-infant interactions [11, 15, 17], reduced sensory cues given by the underfed young that normally elicit maternal behavior [12, 29, 33, 34], and behavioral changes in the mother [7, 24, 28, 34]. Few attempts have been made to separate the influence of sensory stimuli issuing from the mother and littermates from that of nutritional factors on the later behavior of rodents [14].

The maternal response is an adaptive behavior that can be elicited in virgin females by repeated exposure to newborn rats, and which normally is manifested during the perinatal period by lactating rats and mice [5,21]. In an attempt to differentiate the long-term effects of environmental stimuli from those of nutrition, the maternal behavior of adult female rats which were neonatally well-fed or undernourished was investigated. The latter were deprived of food either by daily separation from their mothers and littermates (Experiment 1) or by the nipple-ligation of their mothers (Experiment 2) during the lactation period [14]. Thus, Experiment 1 explores the combined effects of early food and sensory deprivation on subsequent maternal responsiveness, while Experiment 2 attempts to minimize the effects of sensory deprivation.

EXPERIMENT 1

METHOD

Animals

Thirty-two female Wistar rats and their first litters (culled to 8 pups each on postpartum day 1), housed in wire mesh maternity cages (20×40×16 cm), served as subjects. Sixteen of these mothers had been neonatally undernourished by daily removal from the nest along with half of the litter (4 out of 8 pups) to an incubator maintained at 29°C for 12-hr (0800–2000) from postnatal day 1–23. These mothers were marked when pups by clipping a digit on the right fore-paw. The other 16 mothers constituted the control group and were among those pups that had remained in the nest with their mothers and littermates from postnatal day 1–23, except for a daily 3-min period when they were placed in the same incubator as the experimental group. The control and experimental females were chosen at random from a total of at least 40 litters that had been routinely standardized to 8 pups per mother on postpartum day 1.

TABLE 1

BODY WEIGHTS (±SEM, g) OF PUPS BETWEEN 1 AND 20 DAYS OF AGE OF CONTROL AND NEONATALLY UNDERNOURISHED FEMALE RATS

Age (Days)	Control	Undernourished	Number of Animals n	F (df)	p^*
		Experiment 1			
1	5.23 ± 0.10	5.16 ± 0.12	52	0.074 (1,24)	0.783†
5	8.91 ± 0.26	6.89 ± 0.14	52	15.095 (1,24)	0.009
10	14.01 ± 0.47	12.67 ± 0.52	52	0.988 (1,24)	0.668†
15	21.03 ± 0.51	18.30 ± 0.63	52	3.753 (1,24)	0.061†
20	26.18 ± 0.63	22.53 ± 0.75	52	5.008 (1,24)	0.032
		Experiment 2			
1	4.59 ± 0.06	4.56 ± 0.07	48	0.059 (1,10)	0.806†
5	9.66 ± 0.16	7.78 ± 0.21	48	9.842 (1,10)	0.010
10	15.79 ± 0.36	11.98 ± 0.42	48	7.560 (1,10)	0.019
15	22.09 ± 0.32	15.25 ± 0.33	48	31.759 (1,10)	0.0004
20	29.04 ± 0.29	18.17 ± 0.53	48	51.060 (1,10)	0.0001
	(Experiment 1 Undernourished vs. Experiment 2 Undernourished)				
1	5.16 ± 0.12	52	4.56 ± 0.07	48	2.625 (1,17)	0.121†
5	6.89 ± 0.14	52	7.78 ± 0.21	48	3.064 (1,17)	0.096†
10	12.67 ± 0.52	52	11.98 ± 0.42	48	0.185 (1,17)	0.675†
15	18.30 ± 0.63	52	15.25 ± 0.33	48	2.918 (1,17)	0.102†
20	22.53 ± 0.75	52	18.17 ± 0.53	48	3.841 (1,17)	0.064†
	(Experiment 1 Control vs. Experiment 2 Control)					
1	5.23 ± 0.10	52	4.59 ± 0.06	48	11.759 (1,17)	0.003
5	8.91 ± 0.26	52	9.66 ± 0.16	48	1.309 (1,17)	0.267†
10	14.01 ± 0.47	52	15.79 ± 0.36	48	1.782 (1,17)	0.197†
15	21.03 ± 0.51	52	22.09 ± 0.32	48	0.961 (1,17)	0.656†
20	26.18 ± 0.63	52	29.04 ± 0.29	48	7.419 (1,17)	0.015

*p Values were calculated using a 2 way ANOVA.
†Not significant difference.

The two groups were weaned and weighed at 25 days of age, after which they were allowed free access to water and food (Purina chow). The females were kept in groups of 4–5 until reaching 110–120 days of age when they were mated with neonatally well-fed male rats of similar age, and subsequently tested for maternal behavior. All subjects were kept in an air-conditioned colony room maintained at a constant temperature and humidity, and a 14-hr light (0700–2100)/10-hr dark (2100–0700) illumination cycle. Food and water were available ad lib.

Procedure

Approximately 3 days before parturition, nulliparous females were placed individually in wire-mesh maternity cages (20×40×16 cm) provided with forty paper strips (ca. 12 g) as nesting material. Water and food were placed in the front of the cages and, in all cases, the female chose either of the back corners of the cage as her nest site and used the paper strips to construct a nest. Daily 10-min observations of maternal behavior were carried out between 1000 and 1200 hr from day 1–21 of the lactation period. The observer remained quietly seated in the animal room, approximately 1.5 m from the cage, and scored visually the duration of nursing, and the frequency of self-grooming and circling by the mother. Nursing and grooming generally appeared independently; however, when they were concurrent both the number of grooming bouts and the nursing duration were scored. Circling behavior was not observed concurrently with any other behavior. A nest rating was made immediately following each 10-min observation period. The first of the 21 daily observations began within 16-hr after birth (first observation=postpartum day 1). A retrieving test was performed on days 4, 8 and 12, after the nest rating had been made at the end of the 10-min observation period. This test was performed on only three days, to limit interference with mother-infant interactions. For the test, the mother was taken out of the home cage and all pups were removed 10–15 cm from the nest, and then the mother was replaced on the empty nest.

Behavior

The behaviors studied were categorized as follows:

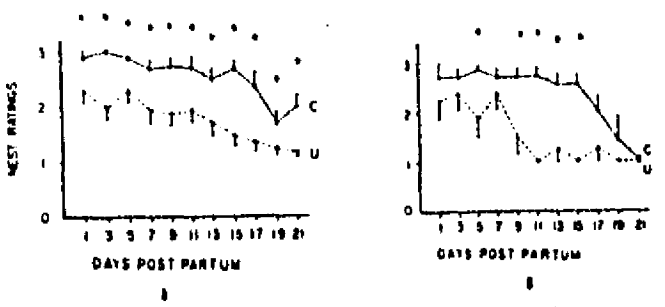

FIG. 1. Mean nest ratings (±SEM) obtained from lactating control (C) and neonatally undernourished (U) mothers. Scoring was made as follows: 3, round well-made nest of approximately 3–4 cm in height; 2, partially destroyed oval-shaped nest; 1, nearly destroyed nest of amorphous shape. Undernourishment was carried out by (A) daily separation from the mother and littermates or (B) nipple-ligation of the mother. Asterisks indicate days on which differences between U and C mothers were statistically significant, ($p<0.05$).

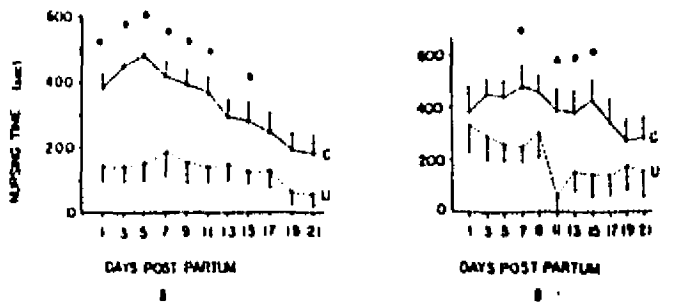

FIG. 2. Mean nursing time (±SEM) in seconds of lactating control and neonatally undernourished mothers observed over a 10-min period. See Fig. 1 for experimental conditions and symbols.

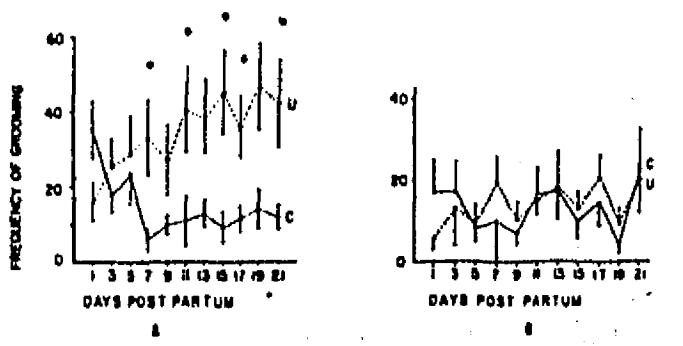

FIG. 3. Mean frequency (±SEM) of self-grooming (face washing, fur licking, scratching) by lactating control and neonatally undernourished mothers observed over a 10 min period. See Fig. 1 for experimental conditions and symbols.

Nest rating. This was scored daily on the basis of the shape and dimensions of the nest. A score of 3 was assigned to a round well-made nest of approximately 3–4 cm in height; 2, to a partially destroyed oval nest; and 1, to a nearly destroyed nest of amorphous shape. The nest ratings were made without disturbing the mother and her litter.

Nursing time. Nursing behavior was defined as the maintenance of a crouching posture on the part of the mother over the pups, permitting them easy access to her nipples for suckling. The female usually had her hindlegs stretched out over the pups and her back arched.

Frequency of grooming. The frequency of self-grooming bouts defined as face washing, fur licking, and scratching was recorded daily during the 10-min observation period.

Circling behavior. The frequency of the initiation of circling behavior (walking or running in a circle while trying to bite or reach the tail for a total of 3–20 complete rounds until the tail was held in the mouth and then rested on the nest) was recorded during the 10-min observation span.

Retrieving latency. The latency of retrieval was defined as the time which elapsed between the mother being placed on her empty nest and the retrieval of the first of her pups.

Because little variation was observed in the values of the nest-rating, nursing time, grooming and circling from one day to the other, it was arbitrarily decided to analyze each behavior every other day (days 1, 3, . . . 21). These scores were compared in a 2 (Nutritional Regimes) × 11 (Days) ANOVA with repeated measures on time [35]. A similar analysis of variance, 2 (Nutritional Regimes) × 3 (Days) was computed for scores of retrieving latency. Additionally, a one-way analysis of variance of behavioral data on each day postpartum was also performed. Fifty-two pups of each experimental group were initially selected at random and were weighed on day 1 and every five days thereafter. These animals were marked for identification by ink-coloring a part of the nape. Body weights were analyzed on each day with a three-factor "nested" analysis of variance with offspring "nested" within litters within treatment groups.

RESULTS

At weaning undernourished females to be mated in adulthood exhibited significantly lower body weights (mean±SE: 31.91 g±0.63) compared to their controls (49.61 g±0.89), $p<0.001$ (t-test). Although the weights of these females were not recorded at the time of mating in the present experiment, we have observed a significant reduction in the weight of adult male rats undernourished by the same procedure used in other experiments [23]. On postnatal Day 1, the body weights of the pups from the previously undernourished and control mothers were very similar (Table 1), indicating that they differed little at the time of birth, though the weights of their mothers may have differed. Although not systematically determined, it appears that there were no differences in the number of pups born to the two groups of mothers.

Pups from early undernourished mothers had a tendency to weigh less than offspring of normally fed rats throughout the experimental period (Table 1). However, the analysis of variance on each day revealed that body weights were significantly lower only on days 5 and 20, $F(1,24)=15.095$, $p<0.0009$ and $F(1,24)=5.008$, $p<0.032$ respectively. This tendency to reduced weight of the pups might suggest a deficiency in the maternal care of early undernourished dams that stunted the growth of the young.

Analysis of the mean nest-rating scores of early undernourished and control females yielded the main effect of Nutritional Regime, $F(1,30)=57.32$, $p<0.000002$. Additionally, no significant interaction was observed. The analysis of variance in each of the days of study revealed significantly lower nest ratings ($p<0.05$) for early underfed mothers (Fig. 1A).

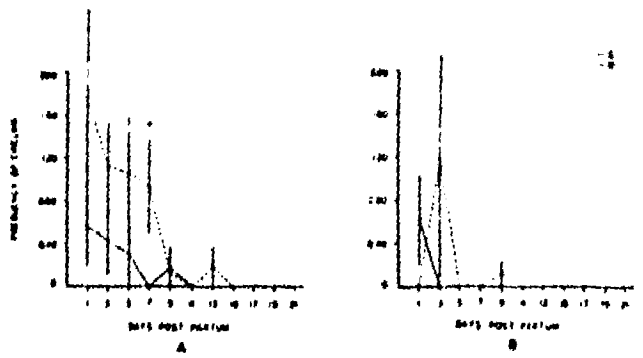

FIG. 4. Frequency of circling movements recorded over the lactation period made by control and neonatally undernourished mothers. See Fig. 1. for experimental conditions and symbols.

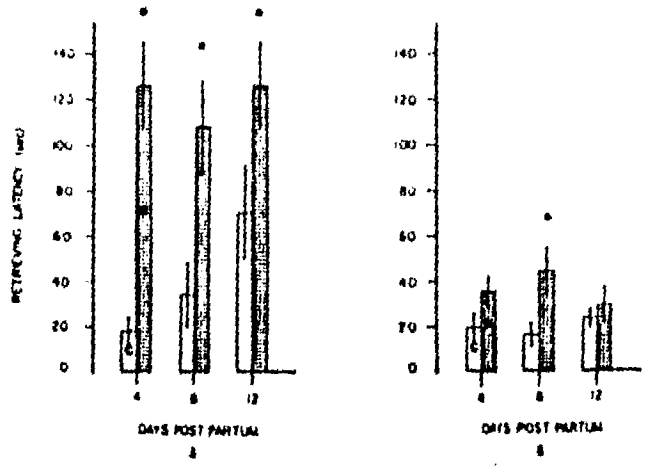

FIG. 5. Mean latency (±SEM) in seconds, for the retrieval of the first pup after placement of the mother on an empty nest, exhibited by lactating control and neonatally undernourished dams. See Fig. 1 for experimental conditions and symbols.

Control mothers spent more time nursing than did early underfed mothers, $F(1,30)=60.63$, $p<0.000002$ (Fig. 2A). No significant interaction was observed. Time spent nursing by early underfed mothers changed little over the first 17 days of lactation, whereas that of control mothers increased between days 1 and 5 and then decreased markedly. Nursing time differed significantly between groups on each of days 1, 3, 5, 7, 9, 11, and 15, but not thereafter.

Mean grooming scores are given in Fig. 3A. Group differences were statistically significant, $F(1,30)=11.68$, $p<0.002$, with early underfed scores high, control scores low. Moreover, a significant Nutritional Regime × Days interaction, $F(10,300)=1.82$, $p<0.05$ was observed. Analysis of variance at each day during lactation revealed that grooming behavior of underfed mothers did not differ from control mothers during the early days postpartum, but the underfed dams groomed significantly more ($p<0.05$) on Days 7, 11, 15, 17 and 21 postpartum (Fig. 3A).

Circling behavior has not been reported in previous studies of early undernutrition and maternal behavior to our knowledge. This stereotyped pattern of movements was manifested by 14 of the 16 neonatally undernourished mothers and by 4 of the 16 normally fed dams. When present, this activity continued for 5–30 sec, with a total of 3–20 complete circular movements. This behavior appeared on days 1–13 in early underfed rats and peaked on day 1, while in the control rats it was manifested on days 1–5 and on day 9 with less intensity and duration (Fig. 4A). Analysis of the mean number of circular movements per day yielded a main effect of Nutritional Regime, $F(1,30)=6.65$, $p<0.014$. No significant interaction was observed. The analysis of variance on each day of the study revealed significant differences ($p<0.02$) only on Day 7 postpartum.

Early undernourished mothers were slow in retrieving pups when compared to control mothers, $F(1,30)=15.32$, $p<0.00074$. No significant interaction was observed. The analysis of variance on each day of the study showed that early underfed rats were significantly ($p<0.02$) slower to start retrieving each day (Fig. 5A).

EXPERIMENT 2

While the previous experiment indicates the presence of severe alterations in the maternal behavior of mothers neonatally deprived of food and sensory stimulation, it is difficult to determine to what extent they are due to the long-term effects of early food restriction, neonatal sensory deprivation, and/or altered patterns of behavior exhibited by pups reared by such mothers which affect the maternal performance [20,34]. Nipple-ligation of the mothers, known to minimize the adverse effects of sensory deprivation inherent in other underfeeding techniques [14], was used in this experiment to undernourish infant rats.

METHOD

Subjects

Eight of the 16 Wistar rats (100–120 days of age) used in this experiment came from 2 paired litters that had been undernourished during the early postnatal period by means of the subcutaneous nipple-ligation of one of the mothers [14]. The paired litters were alternated between the nonligated and ligated mothers every 12-hr, from postnatal day 1–23. Pups were marked by the right ear clipping procedure. The eight controls also came from 2 paired litters that were alternated between two nonligated mothers. The females from these litters were weaned, reared, and mated in the colony room as in Experiment 1. The litters in both the parental and filial generations were standardized to 8 pups.

Procedure

The procedures used to assess maternal behavior were identical to those used in Experiment 1. The 48 pups in each group were weighed as in Experiment 1.

RESULTS

At weaning, the early underfed females (later to be mothers) weighed significantly less (25.15 g± 1.19) compared to their controls (51.15 g±1.76) $p<0.001$ (t-test). The analysis of variance performed on each of the days of body weight determination showed that pups from early underfed dams weighed significantly less ($p<0.05$) than pups raised by normal mothers from Day 5–20 postpartum (Table 1). The comparison of body weights between offspring of formerly undernourished animals of Experiment 1 and 2, did not reveal significant differences (Table 1). Moreover, the com-

parison of body weight measurements between controls of Experiment 1 and 2 showed significant differences only on Days 1 and 20 postnatally, $F(1,17)=11.759$, $p<0.003$ and $F(1,17)=p<0.015$ respectively (Table 1).

The analysis of the mean nest-rating scores revealed a significant main effect of Nutritional Regime, $F(1,14)=26.99$, $p<0.0002$, and a significant Nutritional Regime × Days interaction, $F(10,140)=3.95$, $p<0.0002$. Overall, control mothers made better nests. The interaction component is explained by the finding that the nest ratings of the two groups were similar early in lactation but that those of early underfed mothers started to deteriorate about a week before those of control mothers (Fig. 1B), resulting in significant differences between groups only on Day 5 and Days 9–15 postpartum ($p<0.05$).

The significant main effect of nursing time of Nutritional Regime, $F(1,14)=29.48$, $p<0.0002$, indicates that neonatally food deprived rats spent less time feeding their young. The Nutritional Regime × Days interaction was not significant. The analysis of variance on each day of the study showed significant differences on Day 7 and Days 11–15 postpartum ($p<0.05$) (Fig. 2B).

Underfed dams when compared to their controls did not show significant differences in grooming throughout the experimental period. Additionally, no interaction was observed. The analysis of variance in each of the days of the study did not show significant differences (Fig. 3B).

The mean number of circling movements of early underfed dams was indistinguishable from controls throughout the experimental period (Fig. 4B). No significant interaction was detected. The analysis of variance in each of the days of the study did not reveal significant differences.

Analysis of the retrieving latency scores indicated that early underfed mothers were slow when compared to the controls, $F(1,14)=6.88$, $p<0.01$, throughout the experimental period. There was no significant interaction. Additionally, the analysis of variance in each of the days of lactation revealed significant differences ($p<0.05$) only on Day 8 postpartum (Fig. 5B).

GENERAL DISCUSSION

The results of Experiment 1 indicate that early undernutrition by separation of pups from the mother and littermates has long-lasting effects on the maternal behavior of the progeny, consisting of a reduction in nest rating and nursing time, and lengthy retrieval latencies. Although studies have consistently shown that acute malnutrition of mother rats during the gestation and/or lactation period (sec) severely interferes with maternal responsiveness [7,15], we have presented evidence of altered maternal behavior in well-fed adult female rats that were underfed only during the early postnatal period.

Many of the short and long-term effects of early malnutrition appear to be produced by both nutritional and nonnutritional factors, including the concentration of protein or essential fatty acids in the diet, the amount of handling, and the mother-infant relationship [8, 18, 20, 28]. The procedure used in Experiment 1 to undernourish dams early in life combined both food restriction and sensory deprivation. The latter alone has been shown to interfere with emotional [2] and exploratory [13] responses. Our data support the hypothesis that the effect of food and sensory deprivation are potentiated when they are simultaneously present [13]. To our knowledge, no studies have been carried out on the effects of early undernutrition on later maternal behavior in lactating rats. It has been demonstrated that virgin females that were subjected to protein deficiency in early life show a reduction in both the number of contacts and total time spent with pups, and long retrieving latencies [9]. However, due to differences in the endocrinological states of females, procedures used to undernourish the infant rats, and tests for maternal responsiveness, it is difficult to compare those results with ours.

The finding of a high level of grooming behavior in the neonatally malnourished mothers in Experiment 1 is consistent with studies showing exaggerated self-grooming in underfed mothers concomitant with the acute period of food restriction [7,15]. Similar behavior has also been observed in malnourished nonlactating adult animals [23]. A tendency to show high levels of grooming was exhibited by the control rats in both Experiments 1 and 2 during the first day of the lactation period, perhaps related to the licking of membrane and removal of liquids from the genital and abdominal regions, behavior that normally accompanies birth. Thus, our results suggest that neonatally well-fed rats are initially more active in cleaning the genital and ventral abdominal regions than early underfed dams. Whatever the causes of self-grooming, it is a persistent and compulsive behavior that appears to compete with the maternal responsiveness of the neonatally undernourished mothers.

The compulsive circling behavior that was displayed by females that had been underfed during early infancy by removal from the nest was unexpected. This type of activity has been observed in dogs that were sensorially deprived early in life [31], and also in rats without previous hoarding experience [32], or in response to chemical or electrical stimulation of the brain [4,19]. The neonatally undernourished dams in Experiment 1 appear to be prone to this response, which is interesting in view of the reported heightening of nervous system excitability in malnourished rats [6,24]. Several studies in both man and laboratory animals have suggested that the temporal lobe structures are very sensitive to severe perinatal undernutrition, manifested by EEG abnormalities [23,30]. However, the greater incidence of circling and grooming responses are probably primarily related to early sensory deprivation, since low levels of these behaviors were observed in rats that had been neonatally undernourished by the nipple-ligation technique (Experiment 2) which minimizes sensory deprivation.

Sensory stimulation plays a fundamental role in normal brain growth and in the acquisition of long-term behavioral responses [1, 10, 26]. The procedure used in Experiment 2 may be effective in at least partially dissociating the factors of nutrition and sensory stimulation, which cannot be done with other methods of postnatal undernutrition. However, if neonatal malnutrition impedes the central and peripheral neurophysiological development of the newborn [3, 16, 22, 25, 27], it is unclear how much the sensory input of the undernourished pup is reduced, and thus to what extent this sensory deprivation is involved with impaired maternal performance.

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Undernutrition Induced by Early Pup Separation Delays the Development of the Thalamic Reticular Nucleus in Rats

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A Golgi–Cox study was conducted in neurons of the reticular and lateralis thalamic nuclei in normally and early undernourished Wistar rats at 12, 20, and 30 days of age. In a total of 630 neurons the cell body and the dendritic field areas, as well as the number of dendritic prolongations from camera lucida drawings were quantitated. A general and significant reduction in most reticular thalamic nucleus measurements of early-food-deprived rats was observed compared with control littermates. Additionally, reticular thalamic cells in both normal and neonatally underfed rats exhibited a progressive decline, particularly in cell body area with increasing age. In contrast, the lateral thalamic nucleus did not show significant differences between groups when similar neuronal measurements were carried out. The reticular thalamic nucleus is normally related to the control of sensory afferent transmission, and early food deprivation interferes with the growing process of this nucleus. Therefore the present data support the hypothesis that noxious perinatal environmental influences may result in a maturational deficiency of central nervous system modulatory mechanisms.

INTRODUCTION

Studies on early food restriction and neonatal thyroxine administration in the rat have suggested interference by these factors with the development of possible central modulatory mechanisms in sensory afferent transmission. Evidence stems from increased amplitude of both spontaneous and provoked cortical electrical activity, increased duration of repetitive afterdis-

Abbreviations: LN, TRN—lateral, reticular thalamic nuclei.

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charges elicited by sciatic nerve stimulation (19, 20, 22), decrease of the threshold to provoke convulsive seizures (6, 29), and prolongation of both spontaneous and provoked mitral cell electrical responses to tetanic stimulation (17).

Morphological studies suggested that the thalamic reticular nucleus (TRN) might exert a modulatory influence on a large number of both specific and nonspecific thalamic nuclei (23). Subsequent morphophysiological studies provided evidence for an inhibitory influence from the TRN on the neurons of the dorsal thalamus, particularly those related with the control of visual spatiotemporal afferent messages travelling to the visual cortical area (8, 9, 13, 18, 28, 33). Furthermore, additional anatomic findings showed that TRN neurons project to essentially all thalamic nuclei, producing a powerful inhibitory influence on the transmission of sensory afferent messages (9, 13, 18).

Little is known about the role of various perinatal influences on the development of central nervous system structures underlying the ascending sensory information. This report investigated the effects of early food restriction on maturation of the neurons of the reticular and lateral thalamic nuclei in the rat.

MATERIALS AND METHODS

Timed pregnant Wistar rats were housed individually in breeding cages with food and water *ad libitum* in a temperature-controlled room (24 ± 1°C). At birth animals of either sex were grouped into litters of eight pups per mother by adding or removing extra male pups. Neonatal undernutrition was produced by transfering daily one-half of the litter ($N = 4$) from the nest to an incubator maintained at 29°C (0800 to 2000) for 12 h from postnatal days 1 to 23. In all cases underfed pups ($N = 8$) were placed together inside the incubator, so they were able to maintain certain type of physical interaction during the 12-h period of isolation. The control group consisting of the other half of a litter ($N = 4$) remained in the nest with the mother during postnatal days 1 to 23, except for a daily 10-min period when they were transferred to the same incubator as the experimental group (22). This maneuver was done in order to submit the control pups to the unavoidable handling effects endured by early-underfed rats because of the experimental procedure. Animals were weaned at 25 days of age after which they had free access to water and food. All subjects were in an air-conditioned colony room at constant temperature and humidity, and a 14-h light (0700 to 2100):10-dark (2100 to 0700) illumination cycle. Eight subjects per group (four controls and four experimentals) were weighed and deeply anesthetized with ether, then intracardially perfused with saline and 10% neutral buffered Formalin at 12, 20, or 30 days of age. After 12-h fixation, the spinal cord was

severed at the upper end of cervical segment C1 and the entire brain was removed. Coronal sections (100 to 150 μm) from the diencephalon were stained using the Golgi–Cox procedure.

Location and identification of neural structures were based on the Sherwood and Timiras Atlas (26). Anterior–posterior coordinates to localize the TRN corresponded to 4.1 to 1.6, 5.3 to 2.6, and 5.9 to 3.8 mm; for the LN coordinates corresponded to 3.2 to 2.0, 4.2 to 2.9, and 5.0 to 3.8 mm from the younger to the older experimental groups, respectively. Slides were coded and camera lucida drawings of each neuron were obtained at ×675. Sixty complete and well impregnated neurons were selected in each experimental condition and group of age as follows: 15 cells per rat were examined and quantitated, 5 each from the dorsal, lateral, and ventral portions of the TRN. Concerning LN neuronal sampling, 45 complete and well impregnated neurons per experimental condition and group of age were examined in the same rats used for TRN measurements. Cell body and dendritic field areas were measured with a Ladd graphical digitizer. Dendritic field extension was obtained by measuring the area enclosed by a line traced at the tip of all dendritic prolongations. Additionally the number of primary, secondary, and tertiary dendritic branches measured in the camera lucida drawings were also noted. No attempt was made to correct the compression of the three-dimensional dendritic tree to a two-dimensional sketch. According to other studies the relative differences between neurons remain basically constant when transformed from three to two dimensions (3, 27).

Under ether anesthesia, two additional rats per age group and treatment were intracardially perfused with 10% buffered Formalin, their brains were serially sectioned in the frontal plane, at a thickness of 60 to 80 μm. The sections were stained (Nissl method) and used for recognition, extension, and general morphology of the TRN and LN. Statistical significance between experimental groups and ages were compared in a 2 (nutritional regimes) × 3 (days) ANOVA with repeated measures on time (32). When statistically pertinent, t tests were also carried out for each day postpartum. Statistical differences between ages on cell body and dendritic field areas and experimental conditions were determined using Scheffe's test. Additionally, statistical comparisons on the number of dendritic prolongations were done with Mann–Whitney U tests.

RESULTS

Quantitative analysis of mean cell body area indicates than TRN neurons of early-underfed rats had undergone a significant reduction compared with the mean values of control cell body areas (Table 1). In addition, a significant decline in this parameter was also observed with age in both experimental conditions, although the reduction was evident mainly from 12 to 20 days of

TABLE 1

Analysis of Variance of Mean Cell Body and Dendritic Field Areas in Reticular (TRN) and Lateral Thalamic Nuclei (LN) Neurons of Control and Early-Undernourished Rats at 12, 20, and 30 Days of Age

Measurements	Hypothesis	Calculated F	Significance
TRN, mean cell body area ($N = 360$)	Nutritional regimes (Factor A)	26.97	<0.00002
	Ages (Factor B)	19.55	<0.00001
TRN, mean dendritic field ($N = 360$)	A	16.15	<0.0002
	B	2.44	NS[a]
LN, mean cell body area ($N = 270$)	A	0.10	NS
	B	12.87	<0.0004
LN, mean dendritic field ($N = 270$)	A	4.05	<0.0424
	B	1.07	NS

[a] Nonsignificant differences ($P > 0.05$).

age. Comparisons between groups at each developmental age showed lower significant values in early-underfed rats only at 12 and 20 days of age ($P < 0.05$). The analysis of cell body area values (Scheffe's test) at postnatal day 12 vs. 20 and day 12 vs. 30 in controls, and at day 12 vs. 20 postpartum in the underfed group exhibited also significant differences ($P < 0.05$).

Mean cell body area values in LN neurons were not affected by early food restriction throughout the experimental period, although this parameter was significantly affected by age (Table 1). No significant differences between the experimental groups were observed at each developmental age.

Mean dendritic field area measurements of TRN neurons in early-malnourished rats exhibited lower significant values compared with controls (Table 1). Concerning the effect of age on this parameter no significant differences were observed. Comparisons at each developmental age between the experimental groups indicated significant reductions only on days 12 and 20 postpartum. The Scheffe's analysis revealed a significant difference only when the dendritic field values between day 20 vs. 30 postpartum were compared in control rats ($P < 0.05$). No significant differences were observed in the underfed group.

Although the LN mean dendritic field area measurements showed a significant decrease in the undernourished group throughout the days of the study (Table 1), comparisons between groups at each developmental age revealed a significant reduction in underfed rats only at 12 days of age.

The number of dendritic branches of TRN cells exhibited significant reductions ($P < 0.05$) in most ages and types of dendritic extensions in the early-undernourished group (Table 2). However, this effect was particularly

TABLE 2

Mean Number of Dendritic Prolongations per Neuron of the Reticular Thalamic Nucleus during Development

Age	Primary	Secondary	Tertiary	Total
12 days				
Undernourished ($N = 45$)	3.79 ± 0.16	6.96 ± 0.33	7.00 ± 0.52	5.91 ± 0.24
Control ($N = 43$)	5.02 ± 0.24	9.53 ± 0.46	9.40 ± 0.73	7.98 ± 0.34
Significance	$P < 0.0005$*	$P < 0.0001$*	$P = 0.0465$*	$P < 0.0003$*
20 days				
Undernourished ($N = 45$)	4.42 ± 0.17	8.36 ± 0.30	7.56 ± 0.57	6.77 ± 0.27
Control ($N = 43$)	6.20 ± 0.80	9.55 ± 0.38	10.22 ± 0.47	8.39 ± 0.29
Significance	$P < 0.0125$*	$P = 0.0485$*	$P = 0.0057$*	$P < 0.0125$*
30 days				
Undernourished ($N = 45$)	4.11 ± 0.21	6.73 ± 0.32	6.20 ± 0.51	5.76 ± 0.24
Control ($N = 43$)	3.91 ± 0.20	7.67 ± 0.39	9.13 ± 0.59	6.92 ± 0.31
Significance	NS[a]	NS	$P = 0.016$*	$P = 0.0110$*

[a] Nonsignificant differences ($P > 0.05$).
* Significant differences, Mann–Whitney U test.

consistent and statistically significant when the total number of dendritic prolongations was compared ($P < 0.05$). The analysis of the number of dendritic extensions in LN neurons in most ages and dendritic orders did not show significant differences (Table 3).

Body weight comparisons between groups at each developmental age indicated significantly lower values ($P < 0.05$) in early-underfed rats than controls (Table 4).

DISCUSSION

Our data indicate that neonatal undernutrition produced by removing pups from the mother and littermates interferes with TRN development, as evidenced by the statistically significant reductions in the mean values of the cell body area, dendritic field extension, and number of dendritic prolongations. Previous electrophysiologic evidence suggested that the TRN is involved in the modulation of sensory afferent messages ascending to telencephalic structures (8, 28, 33). Our results clearly reveal an interference with the morphologic maturation of the TRN, compared with LN neuronal development. This interference might presumably produce an imbalance in the inhibitory action exerted by the TRN upon other thalamic nuclei for the filtering of spatiotemporal patterns of ascending activation.

The results also show that the mean cell body area and the dendritic field extension in both the control and the undernourished groups have a progres-

TABLE 3

Mean Number of Dendritic Prolongations per Neuron of the Lateral Thalamic Nucleus during Development

Age	Primary	Secondary	Tertiary	Total
12 days				
Undernourished ($N = 45$)	4.13 ± 0.12	7.26 ± 0.22	7.06 ± 0.41	6.15 ± 0.20
Control ($N = 43$)	4.26 ± 0.27	7.73 ± 0.27	8.44 ± 0.35	6.81 ± 0.22
Significance	NS[a]	NS	$P = 0.0158$*	$P = 0.0436$*
20 days				
Undernourished ($N = 45$)	4.00 ± 0.14	7.28 ± 0.25	8.64 ± 0.39	6.64 ± 0.23
Control ($N = 43$)	4.04 ± 0.12	7.44 ± 0.24	7.28 ± 0.34	6.25 ± 0.19
Significance	NS	NS	$P = 0.009$*	NS
30 days				
Undernourished ($N = 45$)	4.40 ± 0.18	7.57 ± 0.26	7.37 ± 0.41	6.45 ± 0.21
Control ($N = 43$)	3.73 ± 0.11	6.64 ± 0.24	8.04 ± 0.36	6.14 ± 0.21
Significance	NS	NS	NS	NS

[a] Nonsignificant differences ($p > 0.05$).

* Significant difference, Mann–Whitney U test.

sive tendency to decline with increasing age, the effect being particularly significant for the mean cell body area from days 12 to 20 postnatally; in addition this occurs from days 20 to 30 postpartum in the mean dendritic field area of controls. These data suggest that during brain growth perhaps a remodelling process of the synaptic connectivity takes place, in order to optimize the TRN outflow. It is known that in rat and kitten the brain stem reticular core exhibits a progressive loss of heteromorphic protospines from postnatal day 11 until 20 days postpartum and longer (10, 25). Similar results have been noted to occur during the maturation of other neural structures

TABLE 4

Body Weights (g) in Control and Early-Undernourished Rats during Development

	Age (days)		
Treatment	12	20	30
Control ($N = 4$)	13.15 ± 0.66	30.72 ± 0.67	55.15 ± 3.61
Undernourished ($N = 4$)	11.02 ± 0.40	16.25 ± 1.33	36.95 ± 1.98
P values*	<0.05	<0.001	<0.02

* Student's t test.

(4), or in the electric multiunit activity of the mesencephalic reticular formation in response to light and sound in the developing rat (30). Because the limited ages chosen in this study, it might be difficult to understand if the smaller cell body size in 12-day-old undernourished rats reflects an effect of early undernutrition or a suppression of cell growth. However, on the view that in both cortical and reticular cells, the transition of protospines to mature spines appears to begin shortly after the 10th postnatal day (10, 24, 25), it is possible that the cell reduction in the present study might be primarily related with early undernutrition. The appearance of a relative specificity of the TRN to control thalamic nuclei discharges toward the cortex (8, 13, 28, 33), the progressive reduction in the subcortical sensory afferent transmission to the TRN as a result of functional specificity, and the maturation of some other afferent modulatory systems upon the thalamus to control the increasing sensory stimuli because of ear and eye opening and increased proprioception, may probably induce this TRN morphologic rearrangement. Neonatal undernutrition apparently does not change this pattern of organization, but it severely interferes with the sequential development of the thalamic substrate (21). The possibility exists of an overfeeding situation in the control pups because of the undernourishment procedure here used might complicate the interpretation of our findings. However, previous studies revealed no significant body weight differences between control male pups and those maintained by part-time use of an incubator, or by randomly pairing nipple-ligated or "aunt" nonlactating dams with normally lactating rats (5, 22). The mechanisms through which early undernourishment impairs TRN maturation are unclear; however, the reduced amount of nutrients, decreased sensory environmental stimuli (7, 16, 22) and hormonal abnormalities accompanying early food restriction (2, 14, 31) might be interfering with the thalamic substrate for the modulation of the sensory afferent messages continuously pouring into the central nervous system.

Brain modulatory action through cortical and subcortical mechanisms is an active and fundamental process, necessary for the integration of a number of complex physiologic phenomena such as habituation, attention, learning, etc. (1, 11, 12). Our experimental data could perhaps be related with the poor physiologic performance commonly observed in subjects suffering from early undernutrition (7, 15, 16, 22).

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Neonatal Undernutrition and Self-Grooming Development in the Rat: Long-Term Effects

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SALAS, M., S. PULIDO, C. TORRERO AND C. ESCOBAR. *Neonatal undernutrition and self-grooming development in the rat: Long-term effects.* PHYSIOL BEHAV 50(3) 567–572, 1991.—The effect of neonatal undernutrition on six different self-grooming components was examined in male rats during the pre- and postweaning periods. Rats underfed by the maternal nipple-ligation procedure before weaning did not exhibit significant score differences in the various self-grooming measurements. In contrast, after weaning they showed a significant increment in the duration of face-washing, head-washing, fur licking and body-scratching. In all cases, the total postweaning self-grooming activity was significantly increased in the formerly underfed rats. Present data suggest that neonatal undernutrition may presumably interfere with the sequential maturational processes of central and/or peripheral mechanisms underlying some components of self-grooming behavior.

Early undernutrition Self-grooming development Rats

IT has been suggested that during brain maturation, the excitatory processes frequently develop earlier than the inhibitory ones (4,7). These studies suggest that structures which mature later may exert modulatory influences upon neural structures developed earlier. Thus it has been suggested that a number of behavioral patterns, such as locomotion, swimming, self-grooming, etc., are largely modulated through different diencephalic and telencephalic mechanisms (3, 7, 22, 30, 33). Moreover, data have also shown that overall levels of behavioral arousal, are regulated by excitatory and inhibitory structures which become efficient during the first month of life in the rat (7, 8, 22, 23).

Among the numerous rodent behavioral patterns, self-grooming is an important component of the repertoire that may play a central role in reducing ectoparasitism (5), it is a means for evaporative cooling (13), for cleaning and maintenance of the pelage (26), it increases with age (15) and in unfamiliar conditions (12,14) and is associated with reproduction (24,28). Therefore, self-grooming may also be a helpful tool to evaluate the effects of perinatal influences (20).

Previous evidence has shown an increase in self-grooming activity in lactationally malnourished rats 100 to 500 days after commencement of nutritional rehabilitation, and in lactating dams underfed early in life (11, 18, 20), suggesting that in the rat, early food and/or social deprivation, among other lacks, may interfere with the ontogeny of mechanisms underlying self-grooming activity.

The aim of this study was to perform a longitudinal analysis of the development of different self-grooming components in both normal and underfed rats in order to detect the components involved in the total self-grooming increment exhibited by neonatally underfed rats.

METHOD

Animals

The subjects were Wistar strain rats (*Rattus norvegicus*) obtained from a colony maintained by the Institute of Investigaciones Biomedicas, University of Mexico. Forty-eight nulliparous rats between the ages of 100–110 days were housed in 6 groups of 8 females with 4 males of similar age, with food and water ad lib. Pregnant rats were removed from the plastic breeding cages and placed in individual maternity cages (35 × 27 × 17 cm) with grill tops, 2–3 days before the estimated date of parturition. The date of delivery was referred to as day 0 of postnatal life. Within 24 h after birth, male pups were randomly redistributed to 32 dams, so that each litter consisted of 8 male pups. The redistribution was intended to balance possible genetic and prenatal biological differences between litters. All rats were kept on a 14-h light:10-h dark (lights on at 0700 hours) schedule in a room thermostatically maintained at 22–24°C. In all cases, the bedding consisted of wood shavings.

The underfed pups used in this experiment ($n = 16$) came from 8 pairs of litters which had been neonatally undernourished by means of the subcutaneous nipple-ligation of one of the dams (18). In all cases, the nonligated and ligated lactating mothers were alternated between each pair of litters every 12 h (at 0800 and 2000 h), from postnatal day 1 to 24. Thus, for the 8 underfed pups of one litter of each pair, undernutrition occurred during the light phase (0800 to 2000 h) and for the 8 pups of the other litter of the same pair, during the dark phase (2000 to 0800 h). Eighty percent of underfed subjects here employed were undernourished during the dark phase. Although underfed rats were

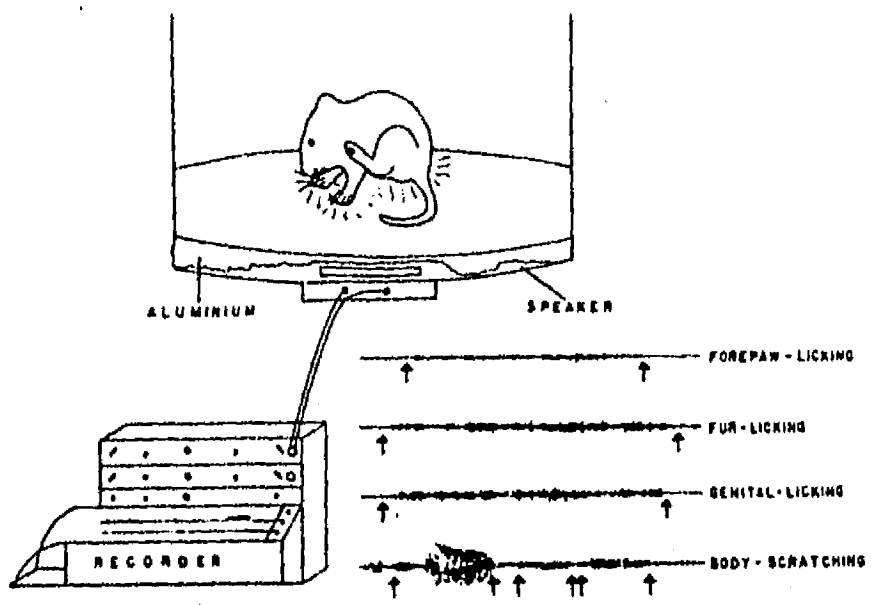

FIG. 1. Procedure employed for the recording of self-grooming components of developing rats. Below to the right, polygraphic records of various self-grooming components (intervals between arrows) measured in a 25-day old rat. Calibration 50 μV and 1 second.

smaller in size and thus easily identified by physical exploration, on postnatal day 26 they were marked for recognition with the right ear-clipping procedure, to differentiate them from the controls that may eventually resemble them when adults. The well fed pups were left unmarked. The controls (n = 16) also came from 8 pairs of litters, alternating 2 nonligated dams between each pair. The two experimental groups were weaned at 25 days of age, after which they had free access to water and food (Purina chow). All subjects were checked when pertinent for ear and eye opening. Animals from these litters with identical treatment were kept in groups of 4–6, and maintained in an air conditioned colony room at constant temperature and humidity, and a 14-h light (0700–2100):10-h dark (2100–0700) illumination cycle. These light:dark cycles were chosen because they resemble the local environment and the experimental conditions used in other studies. In all cases, the same animal was selected from each litter by putting an ink mark on the tail, and tested for grooming activity on days 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and on days 31, 40 and 60 postnatally.

Apparatus

A cylindric stabilimeter was used to measure the activity of rats displayed during the self-grooming bouts. This was constructed with translucent 1 mm plastic and measured 25 cm in diameter and 25 cm in height. The plastic cage was resting on a 25 cm diameter speaker. The opening of the cone of the speaker was covered with a piece of aluminium paper for protection. A single rat was placed on the aluminium paper inside the cage. Thus the displacements produced by the rat during self-grooming bouts modified the surface of the aluminium paper producing sounds which were amplified and recorded on a model 79-C Grass poligraph (Fig. 1). The amplitude of the spikes produced by the rat during the recording sounds before a grooming bout ranged from 0.5–1.0 mm above the baseline. In contrast, during the different self-grooming components, the spike amplitude ranged from 2–20 mm in height (Fig. 1). The stabilimeter cage and the recording equipment were placed in a sound-proof room separated from the ambient noise of the main laboratory. Lighting was provided by a 25-W red lamp located 80 cm above the stabilimeter. In all cases, the isolated room was maintained at 24 ± 2°C throughout the experiments. Food and water were not available for any subjects from 30 min to 1 h before the trial and during the grooming test. At the end of each trial, the animal was returned to its home cage. In order to minimize odors remaining from the previous trial, the cage was rinsed with soap and water and dried following each test. In addition, a fan was turned on to remove surrounding environmental odors.

Procedure

The animal was tested in the stabilimeter by placing it gently in the center of the cage and leaving it undisturbed for a 10-min period. Each time the subject exhibited a bout of self-grooming, a continuous 60 cps signal was manually recorded, the body areas towards which self-grooming was directed were simultaneously penciled on records by the experimenter who sat at a distance of 90 cm from the cage. Because underfed rats were smaller than controls, we were unable to blind the observer to the identity of the rats for this part of the experiment. However, the records were assigned a random number to insure that the measurements were blind with respect to the grooming component, age and treatment. Moreover, the observer was also blind to the hypothesis of the experiment, and measurements of the duration of different self-grooming components recorded by the observer in each age and experimental condition were compared with the counts obtained in four animals per group randomly chosen for other experimenter.

Self-grooming was manually measured by direct observation of records and by counting the duration in seconds of each of

TABLE 1

BODY WEIGHT (±SEM g) OF CONTROL AND UNDERNOURISHED RATS DURING DEVELOPMENT

Age (Days)	Control (n = 16)	Undernourished (n = 16)	F(df)	p*
1	7.33 ± 1.93	7.30 ± 0.09	0.01(1,30)	0.90†
5	10.90 ± 0.42	10.16 ± 0.71	2.50(1,30)	0.12†
10	21.31 ± 0.61	15.05 ± 0.37	76.50(1,30)	<0.05
15	31.41 ± 0.78	20.30 ± 0.45	151.00(1,30)	<0.05
20	36.30 ± 0.68	25.75 ± 0.93	82.58(1,30)	<0.05
25	48.08 ± 1.41	40.04 ± 1.37	16.60(1,30)	<0.05
30	71.47 ± 2.09	61.53 ± 2.09	11.26(1,30)	<0.05
40	99.36 ± 2.37	80.30 ± 3.08	24.03(1,30)	<0.05
60	129.26 ± 2.54	96.91 ± 4.07	45.33(1,30)	<0.05

*p values were calculated using a one-way ANOVA.
†No significant difference.

the following components: forepaw-licking, face-washing, head-washing, fur-licking, genital-licking and body-scratching. Behavioral tests were performed between 0900 and 1300 hours. The self-grooming components were defined as follows: forepaw-licking, included licking of the forepaw and claws; face-washing, involved the washing with paws of the snout and face; head-washing included the rubbing of the head with the limbs; fur-licking included the licking of the abdomen or one foot; genital-licking comprised the picking of genital or perianal areas; body-scratching, included the scratching of body and head with fast or slow ipsilateral hindleg movements.

The score differences obtained for all the self-grooming components, nutritional regimes and ages were compared by using a multivariate analysis with repeated measurements (34). Moreover, score differences for each of the self-grooming components were compared in a 2 (nutritional regimes) × 16 (days) ANOVA with repeated measures. Additionally, post hoc Newman-Keuls tests were conducted when pertinent for significant differences between groups. Body weight differences assessed every five days and on days 40 and 60 postnatally were compared with an ANOVA.

RESULTS

On postnatal days 1 and 5 no significant differences between groups on body weight were observed. Thereafter, the weight of the undernourished animals was significantly lower than in controls (Table 1). The underfed animals were not only smaller in size, but their physical development was also slower, and the ear and eye-openings were delayed by 1–2 and 2–3 days, respectively.

The multivariate statistical analysis performed in the six self-grooming components, experimental groups and ages to examine the relationship among them, revealed that the two nutritional regime groups were significantly different (Wilkes Lambda = 0.848 and F = 15.103, $p<0.0001$). This analysis does not provide information about which of the six individual variables contributed to the group difference nor did it show that self-grooming in the underfed group was greater than the controls on any measure, but it does show that underfed and control groups have different self-grooming characteristics. The two underfed groups (starved during the light phase and underfed during the dark part of the cycle) did not differ statistically from one another in the total self-grooming differences, F(1,239) = 0.015, $p>0.05$. Similar results were obtained when their controls were compared, F(1,239) = 1.586, $p>0.05$. Additionally, the body weight of 2 groups of 13 rats each underfed during one of the two phases of the cycle did not exhibit significant differences, F(1,224) = 0.233, $p>0.05$. Similar results were obtained when the controls were analyzed, F(1,224) = 0.272, $p>0.05$.

TABLE 2

CANONICAL DISCRIMINANT ANALYSIS. INDIVIDUAL SELF-GROOMING COMPONENTS WITH STANDARDIZED COEFFICIENTS

Canonical Factors	Standardized Coefficients
Head-washing	.724
Face-washing	.517
Fur-licking	.485
Body-scratching	.483
Genital-licking	.272
Forepaw-licking	.087

The canonical discriminant analysis resulted in significant differences among nutritional regimes ($p<0.0001$), and helped to illustrate that face-washing, head-washing, body-scratching and fur-licking which had a standardized coefficient greater than 0.4, contribute more in the underfed group to self-grooming differences among the groups (Table 2). Moreover, the univariate analysis of variance showed consistently the significant main effect of nutritional regime ($p<0.05$) in underfed rats on all self-grooming components excepting on forepaw-licking. Post hoc Newman-Keuls tests performed in specific days of the study revealed that early underfed rats exhibited increased significant scores over controls ($p<0.05$) (see Figs. 2 and 3).

DISCUSSION

Present evidence indicities that neonatal undernourishment of rats lead to increased self-grooming activity detectable after weaning when rats are returned to a nutritionally balanced diet. This increase in total self-grooming activity is essentially caused by an increase in face-washing, head-washing, fur-licking and body-scratching. In this sense, our findings are partly in line with previous studies showing an increased total self-grooming activity in adult rats underfed early in life (10, 20, 31). Moreover, they provide the additional information that neonatal undernutrition may presumably provoke long-lasting effects upon central and/or peripheral mechanisms subserving some self-grooming components. The observations also indicate that in neonatally underfed rats, self-grooming components develop in a similar sequence to normal rats, with paws, face and head-washing, followed by the appearance of fur, genital-licking and consistent body scratching around the second postnatal week as described elsewhere (27).

The increased self-grooming activity after weaning in nutritionally rehabilitated rats may be analyzed according to the following possibilities: the first is that although the brain circuitry underlying self-grooming components is at present poorly understood, the analysis of the neural substrates including lesions of specific brain regions (3) and neuropharmacological manipulations suggests that the periaqueductal gray is one of the primary sites related to self-grooming stimulated by ACTH, and that the activity of neostriatum and accumbens, via a nigro-colliculus-central gray pathway, may modulate the display of excessive grooming (12,33). The postweaning increase in self-grooming which favors head-washing, fur-licking and body-scratching

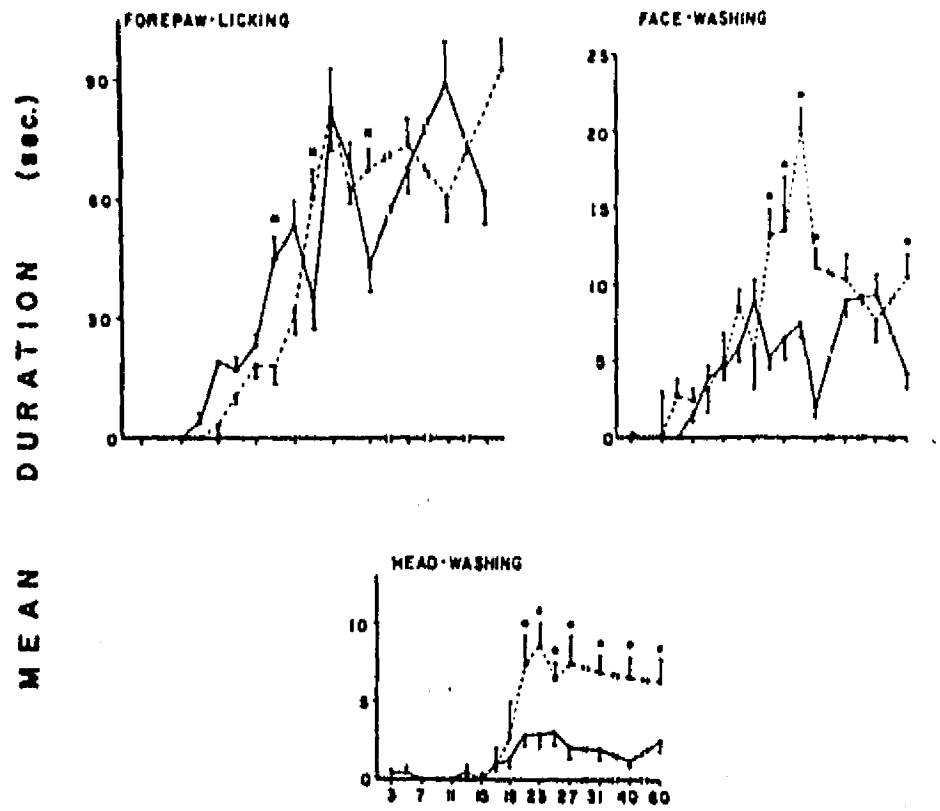

FIG. 2. Mean duration time of different self-grooming component spent by control (solid lines) and neonatally undernourished rats (interrupted lines) during development. Asterisks in this and the following figure indicate days on which differences between groups were statistically significant.

activities, observed as a result of early food restriction, may perhaps be the result of an interference with the maturation of the neural structures mentioned above, or others related to motor coordination (2,19).

Another possibility is that the procedure used to underfeed neonatal rats is likely to disrupt the mother-infant-environment interaction, and this may in turn be affecting behavioral development (10, 17, 31, 32). Although the nipple-ligation method minimizes some of the effects of social deprivation, and since both the total self-grooming and some of its component self-grooming were significantly increased after weaning, one may speculate that under our experimental conditions these effects lead a negligible influence. Nevertheless, the role of neonatal social stimulation to prevent the increased self-grooming obtained in isolation, might perhaps be evidenced by assessing self-grooming under conditions of a social environmental context, or a peculiar physiological state, as described elsewhere (22,31).

Moreover, the use of litters constituted only by control or underfed males may promote greater anogential licking from the dams (24), and this in turn may interact with the nutritional manipulation. However, the postweaning increments of some self-grooming components in early underfed rats suggest that the interaction between these two factors is poor. The specific location and transitory duration of the anogential maternal licking may perhaps produce lasting consequences upon other behavioral patterns (25).

Another explanation is that after weaning, grooming bouts of early underfed rats following food intake might be higher than those of normally fed rats because of an increased postprandial thermogenesis, and as a mechanism for temperature control (13). Although the factors involved in heat loss and production in rats are still unclear, the experimental evidence available shows that underfed adult rats during acute or chronic malnutrition (16), and that neonatally undernourished rats during rehabilitation, do not increase their food intake (29). Besides, early underfed male rats at the end of a 3-week period of undernutrition, and after 9 days of nutritional rehabilitation, had lower rectal temperatures than controls, but subsequent measurements at 45 and 90 days revealed no significant differences (29). Additionally, neonatally underfed rehabilitated rats also exhibited elevated resting oxygen consumption and greater thermogenic activity after noradrenaline adminstration, suggesting that their homeothermy results from a greater heat production followed by an increased heat loss (29). The increased heat loss might be promoted by the low fat content, and by the poor development of the pelage, as evidenced by the atrophy of epidermis, and the reduction in the thickness and length of the hairs observed in neonatally undernourished rats (21) and in adult underfed and rehabilitated pigs (6).

Moreover, it has been suggested that the increased self-grooming of underfed rats might be the result of possible differences in the care of the body pelage because of the rough handling related to their hyperemotionality, and differences in skin irritation due to various levels of skin infection associated to impaired immune response (9). In this study, gross observations in the early underfed rats did not show conspicuous signs

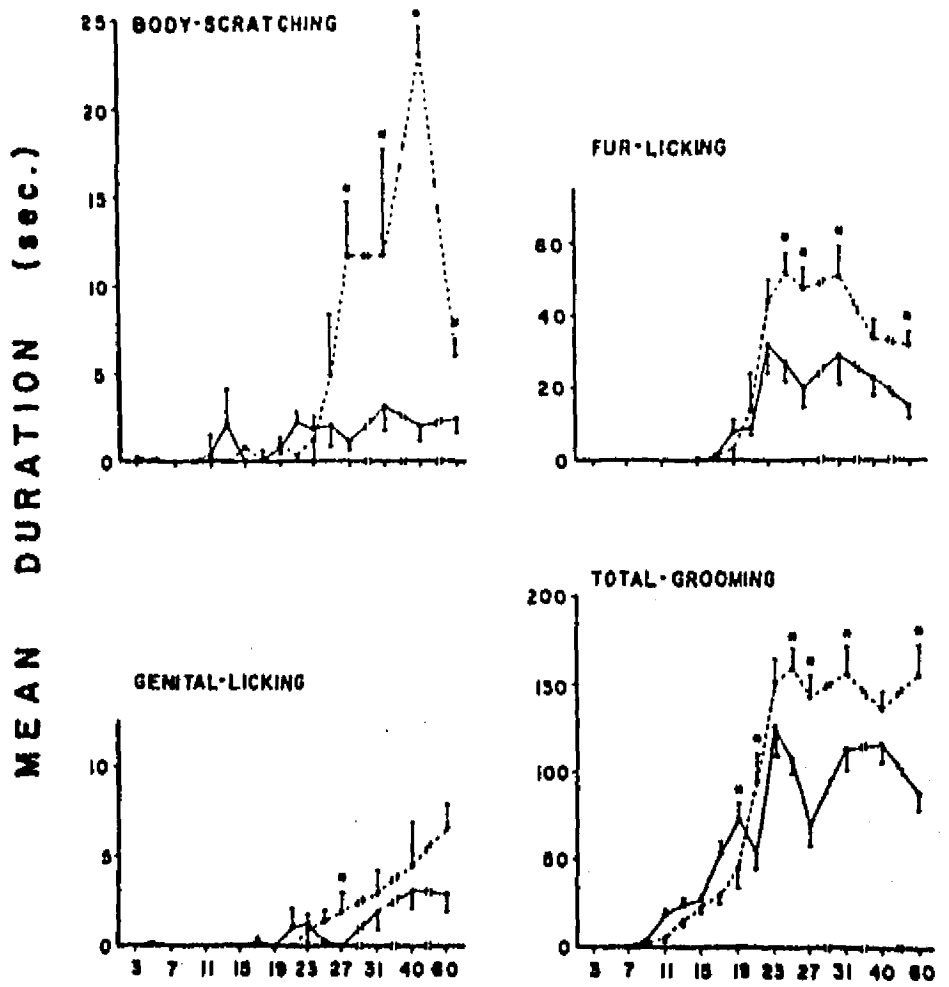

FIG. 3. Mean duration time of the total and several self-grooming components spent by control and early undernourished rats during development. Codes and symbols as in Fig. 2.

of dishovement of the pelage, dirty skin, irritation and/or local infections, and the self-grooming components were selectively affected and not modified as a whole.

Finally, the procedure employed here at weaning to identify early underfed rats may have induced the mothers to give their ear-clipped pups more attention and stimulation than they gave the controls (1), and this in turn could result in the self-grooming differences observed here. However, because the ear-punching has been involved in maturational behavioral effects other than self-grooming before weaning (1), and since increased consistent self-grooming activity has been observed in both marked (31) and unmarked underfed rats (11,20), this explanation seems to be implausible. Although some of these peripheral possibilities appear to be unattractive, further experiments are required to discard their potential influence upon self-grooming.

ACKNOWLEDGEMENTS

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Neonatal Undernutrition and Amygdaloid Nuclear Complex Development: An Experimental Study in the Rat

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This study describes the morphology of neurons from the basolateral (ABL), central (ACE), and medial (AM) nuclei of the amygdaloid complex in neonatally undernourished (U) and control (C) Wistar strain rats. The cells were impregnated with the Golgi–Cox technique and studied at the ages of 12, 20, and 40 days postnatally. In the U-pups the neurons of the three nuclei displayed a reduced somatic area compared to that of the C-group on Days 12 and 20. However, at 40 days, this difference diminished due to a reduction in the somatic area of the C-group. The dendritic area also appeared reduced on Days 12 and 20 in the U-group, but on Day 40 it reached control values. The neurons from ABL and ACE suffered a significant decrease in the number of dendritic branches due to undernutrition, but the AM nucleus did not show this change. The data suggest different vulnerability of these amygdaloid nuclei to neonatal undernutrition. The findings also suggest that the abnormal emotional response characteristic of perinatal undernourished rats could have a morphological cause. © 1993 Academic Press, Inc.

INTRODUCTION

Neonatal undernutrition mainly affects the areas of the nervous structures which undergo a postnatal cell proliferation phase (21, 64) such as the cerebral (5, 35, 64) and cerebellar cortices (4, 10, 27, 59), the hippocampus (1, 15), and the olfactory bulb (2, 13). Moreover, the dendritic trees of these cerebral structures also suffer a reduction in the neuronal dendritic branching and dendritic spines (11, 14, 18, 25, 30, 57), hence, their connectivity is severely affected.

In addition, undernutrition delays the maturation of sensory systems and expression of motor patterns (3). Undernourished rats explore less than controls (10, 39), they are slower and inefficient in acquiring discriminatory learning tasks (6, 9, 26, 51, 61), and the adult female displays a poor maternal response (54, 66).

Associated to these behavioral alterations, early undernourished rats consistently exhibit an abnormal emotional response and have been described as very active (68), more reactive (34), aggressive (23), more responsive (65), and, in the open-field test, they appear more emotional, explore less, and defecate more than controls (19, 53). When exposed to novel or to stressfull situations they also attain higher blood levels of corticosteroids (63, 67).

It has been proposed that inhibitory brain processes involved in modulatory functions mature later than excitatory processes (8, 55). Hence, regulatory systems of early ontogenetic origin may also be vulnerable during the neonatal period due to a late adaptational adjustment to environmental demands. In the early undernourished rat, limbic structures, specifically the amygdaloid complex, could be affected, thus resulting in emotional and visceral response alterations.

It has previously been reported that the temporal lobe structure is particularly susceptible to neonatal undernutrition. Salas and Cintra (52) described a proportion of slow frequency waves in the temporal lobe higher than those in other cerebral zones of undernourished rats and also compared to controls. Additionally, other reports claim morphological alterations and delay in electroencephalographic maturation in the hippocampus (7, 15, 44, 49). Forbes *et al.* (22) found that high stimulus thresholds elicited amygdaloid afterdischarges during an attempt to produce kindled motor seizures in malnourished rats. The latter suggests that undernutrition reduces the capacity of the amygdala to develop changes in its electrical discharge pattern. Presently, no data describing the morphological effects of neonatal undernutrition on the amygdaloid neurons are available to our knowledge in the literature.

This study attempts to ascertain if neonatal undernutrition exerts changes in the morphological structure of the amygdaloid complex. Three nuclei were chosen: the central nucleus (ACE), the basolateral nucleus (ABL) in the basolateral subdivision, and the medial nucleus (AM) in the corticomedial subdivision. Each one is involved with diverse functional systems but all are related to the modulation of emotional and visceral responses associated with novel and stress situations. For

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this investigation brains of control and neonatally undernourished rats were studied at three developmental ages.

METHOD

Animals. Three groups of two adult male and five female virgin Wistar strain rats weighing 250 to 300 g were housed in 50 × 40 × 20-cm cages for mating. They were maintained on an *ad libitum* diet of Purina chow and water, with controlled temperature of 24 ± 1°C and 12 h of light per day cycle (lights on at 0700 h). Two days before delivery each female was placed in an individual acrylic cage of 45 × 30 × 20 cm, with clean shavings for nesting and food and water *ad libitum*. Twelve litters were used for this study. On Postnatal Day 1 the litters were adjusted to eight pups: four females (F) and four males (M) and their body weight was registered. One half of each litter, 2F 2M, was assigned to the undernourished group (U) and the other half served as control group (C). Neonatal undernutrition was produced by daily removing the U-pups from the nest and placing them in an incubator maintained at 29°C for 12 h (0800–2000) from Postnatal Days 1–23. During this period the C-pups remained undisturbed with free access to the dam. This procedure was suspended at the age of 24 days. From then on the U-pups were exposed to nutritional rehabilitation and left to remain in their homecage with free access to the dam, food, and water. In order to distinguish the U-pups from their C-siblings in the nest, their heads were marked with a dot of colored washable ink. Early weaning could damage the U-pups, already weakened by their limited diet, therefore their stay with the dam was prolongued to Postnatal Day 28. At this age all pups were weaned and housed in groups of six to eight rats of the same sex and nutritional treatment.

Surgical and staining procedure. In order to avoid a morphological difference associated with sex, only male pups were considered for this study. On Days 12, 20, and 40, eight pups from each condition were weighed, anesthetized with ether, and sacrificed by decapitation. Their brains were immediately removed and weighed, cut into four coronal blocks, and immersed in a Golgi–Cox solution (50) for impregnation. After 3 weeks, the blocks were dehydrated and embedded in low-viscosity nitrocellulose. Subsequently they were cut in coronal sections of 100 to 150 μm and mounted in serial order. For further identification slides were coded to ensure blind observations with respect to age and experimental condition. Location and identification of neural structures were based on the Sherwood and Timiras Atlas (60). Samples of the ABL, ACE, and AM nuclei were taken from sections corresponding to the anteroposterior coordinates A3.6–2.3 mm for 12-day-old brains, A5.0–3.5 mm for the 20-day-old groups, and A5.3–4.1 mm for the 40-day-old groups.

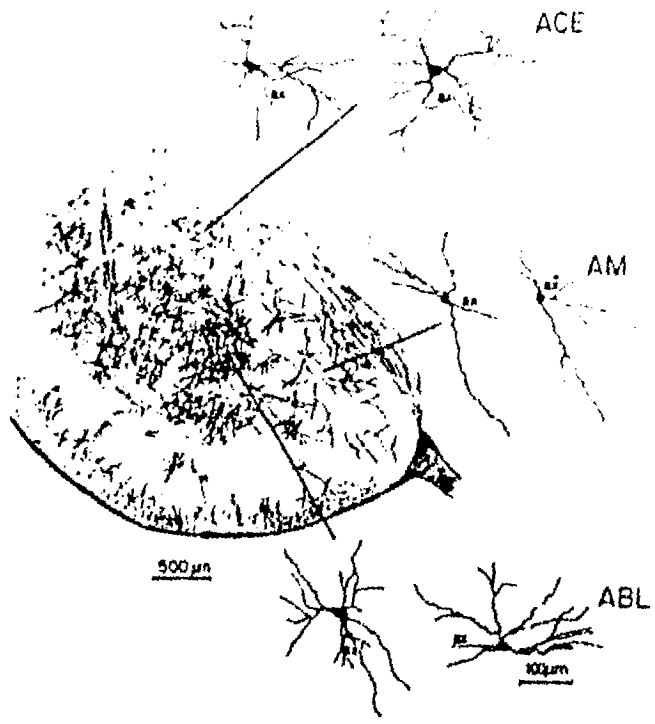

FIG. 1. Coronal section drawing (A 4.4 mm) of the amygdaloid complex in a 40-day-old rat brain, with neurons corresponding to the ABL, ACE, and AM nuclei.

Morphometric analysis. Drawings from complete and well-impregnated neurons (Fig. 1) were obtained with a camera lucida adjusted to a light microscope at 675× magnification. Twenty-five neurons from each age and experimental condition were examined, resulting in a total of 150 neurons for each nucleus. Cell body and dendritic area were measured with a Ladd graphical digitizer. For this purpose, a line was traced connecting the tips of the peripheral dendrites. The drawings were placed on the digital surface and the contour of the resulting area and of the soma were retraced with a fine tip. The dendrites were classified into primary, secondary, tertiary order, and so on considering their place of origin. According to this strategy the number of dendrites was counted.

Statistics. Statistical analysis was based upon the Systat Statistical Package. The values for body and brain weight, for somatic and dendritic areas, and for every dendritic order were analyzed with a two-way ANOVA (nutrition × age). In addition the values for all dendritic orders were analyzed with a MANOVA (nutrition × age × dendritic order). The differences between experimental groups on particular ages were determined with the Newman–Keuls post hoc test.

this investigation brains of control and neonatally undernourished rats were studied at three developmental nges.

METHOD

Animals. Three groups of two adult male and five female virgin Wistar strain rats weighing 250 to 300 g were housed in 50 × 40 × 20-cm cages for mating. They were maintnined on an *ad libitum* diet of Purina chow and water, with controlled temperature of 24 ± 1°C and 12 h of light per day cycle (lights on at 0700 h). Two days before delivery each female was placed in an individual acrylic cage of 45 × 30 × 20 cm, with clean shavings for nesting and food and water *ad libitum*. Twelve litters were used for this study. On Postnatal Day 1 the litters were adjusted to eight pups: four females (F) and four males (M) and their body weight was registered. One half of each litter, 2F 2M, was assigned to the undernourished group (U) and the other half served as control group (C). Neonatal undernutrition was produced by daily removing the U-pups from the nest and placing them in an incubator maintained at 29°C for 12 h (0800–2000) from Postnatal Days 1–23. During this period the C-pups remained undisturbed with free access to the dam. This procedure was suspended at the age of 24 days. From then on the U-pups were exposed to nutritional rehabilitation and left to remain in their homecage with free access to the dam, food, and water. In order to distinguish the U-pups from their C-siblings in the nest, their heads were marked with a dot of colored washable ink. Early weaning could damage the U-pups, already weakened by their limited diet, therefore their stay with the dam was prolongued to Postnatal Day 28. At this age all pups were weaned and housed in groups of six to eight rats of the same sex and nutritional treatment.

Surgical and staining procedure. In order to avoid a morphological difference associated with sex, only male pups were considered for this study. On Days 12, 20, and 40, eight pups from each condition were weighed, anesthetized with ether, and sacrificed by decapitation. Their brains were immediately removed and weighed, cut into four coronal blocks, and immersed in a Golgi–Cox solution (50) for impregnation. After 3 weeks, the blocks were dehydrated and embedded in low-viscosity nitrocellulose. Subsequently they were cut in coronal sections of 100 to 150 μm and mounted in serial order. For further identification slides were coded to ensure blind observations with respect to age and experimental condition. Location and identification of neural structures were based on the Sherwood and Timiras Atlas (60). Samples of the ABL, ACE, and AM nuclei were taken from sections corresponding to the anteroposterior coordinates A3.5–2.3 mm for 12-day-old brains, A5.0–3.5 mm for the 20-day-old groups, and A5.3–4.1 mm for the 40-day-old groups.

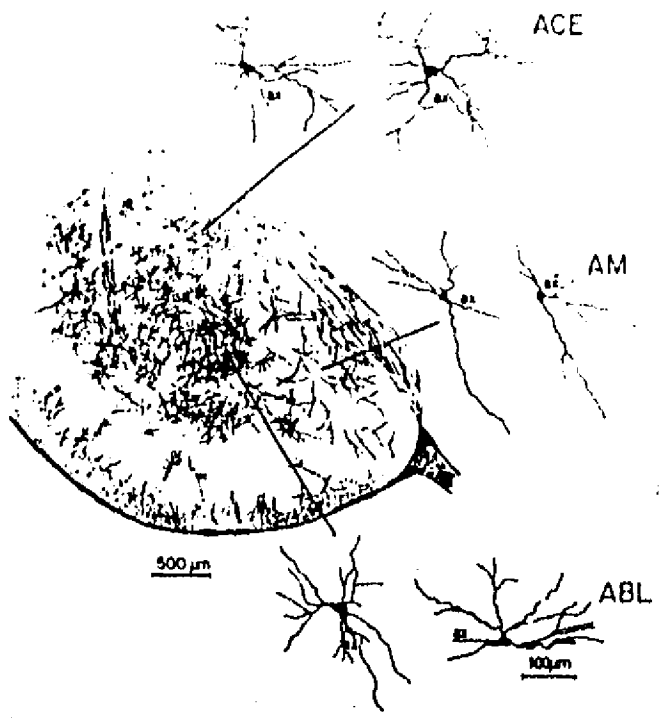

FIG. 1. Coronal section drawing (A 4.4 mm) of the amygdaloid complex in a 40-day-old rat brain, with neurons corresponding to the ABL, ACE, and AM nuclei.

Morphometric analysis. Drawings from complete and well-impregnated neurons (Fig. 1) were obtained with a camera lucida adjusted to a light microscope at 675× magnification. Twenty-five neurons from each age and experimental condition were examined, resulting in a total of 150 neurons for each nucleus. Cell body and dendritic area were measured with a Ladd graphical digitizer. For this purpose, a line was traced connecting the tips of the peripheral dendrites. The drawings were placed on the digital surface and the contour of the resulting area and of the soma were retraced with a fine tip. The dendrites were classified into primary, secondary, tertiary order, and so on considering their place of origin. According to this strategy the number of dendrites was counted.

Statistics. Statistical analysis was based upon the Systat Statistical Package. The values for body and brain weight, for somatic and dendritic areas, and for every dendritic order were analyzed with a two-way ANOVA (nutrition × age). In addition the values for all dendritic orders were analyzed with a MANOVA (nutrition × age × dendritic order). The differences between experimental groups on particular ages were determined with the Newman-Keuls post hoc test.

TABLE 1

ANOVA Analysis for Body and Brain Weight Values of Control and Undernourished Rats during Development

		Body weight		Brain weight	
Factor	*df*	*F*	*P*	*F*	*P*
Nutrition	(1, 42)	40.18	<0.0001	12.33	<0.001
Age	(2, 42)	674.57	<0.0001	249.63	<0.0001
Nutrition × age	(2, 42)	5.46	<0.008	0.30	NS

Note. NS, nonsignificant difference.

RESULTS

There were significant diet effects on body and brain weight for the three age groups studied; also, a reduction in body size was observed and a delay of 1 to 2 days in ear and eye opening. The values of the ANOVA analysis for body and brain weights are shown on Table 1.

In the ABL nucleus most of the neurons were large and multipolar with a pyramidal or pyramidal-like soma. These were equivalent to the class I neurons described elsewhere (41). The somatic and dendritic areas of these cells revealed a significant nutrition and age effect (Table 2). In the U-group the somatic area was notoriously decreased on Day 20 ($P < 0.002$) while this deficiency was minimal on Days 12 and 40 (Fig. 2). On the other hand, the dendritic area was significantly decreased on Days 12 ($P < 0.01$) and 20 ($P < 0.001$) but almost reached control values on Day 40. The number of dendritic branches in the ABL suffered a significant effect associated with nutrition, with age, and with the interaction nutrition × age (Table 3). The effects of neonatal undernutrition were more intense on the first-, second-, third-, and fourth-order branches (Table 3). Age factor also had a significant effect on the first, second, and peripheral dendritic branches; and nutrition × age interaction showed an effect on the fourth-order branches (Table 3). At each age, not all differences between groups were statistically significant, but a constant decrement in the number of dendritic branches was observed in the U-group (Fig. 3). Also, in both groups an increment in the number of dendritic branches was observed from Day 12 to Day 20 and to Day 40.

In the ACE nucleus the majority of impregnated cells were medium-sized neurons with irregular and fusiform soma giving origin to four to six primary dendrites. Both somatic and dendritic areas suffered a significant effect due to nutrition and to the interaction nutrition × age (Table 2). A significant decrease in both somatic and dendritic areas was observed in the U-group on Days 12 ($P < 0.004$, $P < 0.001$) and 20 ($P < 0.001$, $P < 0.004$), but this difference disappeared on Day 40 (Fig. 2). The MANOVA analysis indicated a significant effect due both to the nutritional and to the age factor, but not to the interaction of both factors in the number of dendritic branches of ACE. The main effects due to nutrition were observed in the first-, third-, and fourth-order dendritic branches. The age factor exerted a significant influence upon all the dendritic arborizations with the exception of the sixth-order branches and the interaction nutrition × age had a significant effect only on the first-order branches (Table 3). In both experimental groups an increment in the number of dendritic branches from Day 12 to Day 20 and to Day 40 was observed (Fig. 3) and the values obtained for the U-group were constantly inferior to the values for the C-group, even though not all differences were statistically significant.

The neurons observed in the AM nucleus had small fusiform or pyramidal-like soma, from which two or

TABLE 2

Effect of Neonatal Undernutrition and Age on the Somatic and Dendritic Areas of Three Amygdaloid Nuclei in Rats

	ABL		ACE		AM	
Factor (df)	*F*	*P*	*F*	*P*	*F*	*P*
			Somatic area			
Nutrition (1, 144)	(10.15)	<0.002	(21.95)	<0.0001	(40.10)	<0.0001
Age (2, 144)	(4.45)	<0.01	(2.20)	NS	(14.97)	<0.0001
Nutrition × age (2, 144)	(2.57)	NS	(6.60)	<0.002	(3.11)	<0.04
			Dendritic area			
Nutrition (1, 144)	(13.66)	<0.0001	(31.24)	<0.0001	(10.78)	<0.001
Age (2, 144)	(12.66)	<0.0001	(1.84)	NS	(2.59)	NS
Nutrition × age (2, 144)	(2.25)	NS	(7.47)	<0.001	(1.91)	NS

Note. NS, nonsignificant difference.

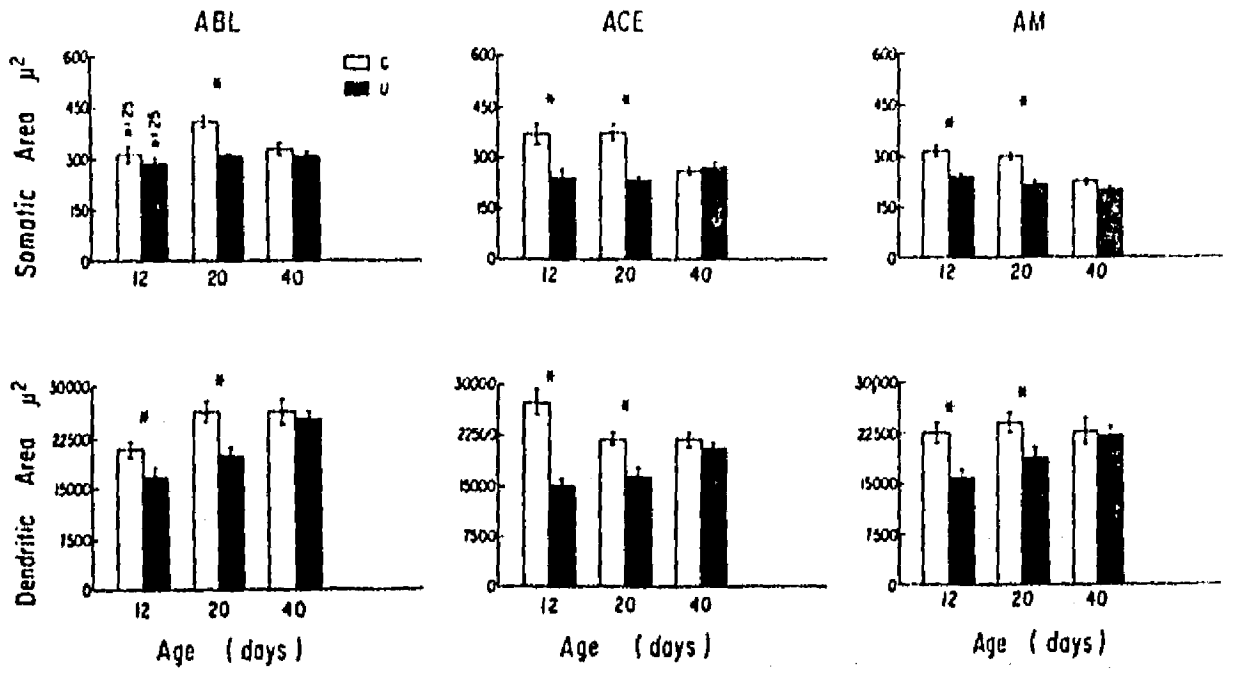

FIG. 2. Mean values ± SEM for somatic and dendritic areas for three amygdaloid nuclei in control and undernourished rats. *Significant difference between groups for every particular age obtained with the Newman-Keuls test.

three large very extended dendrites arose and these depicted scarce branching. The ANOVA analysis indicated a significant effect of nutrition, of age, and nutrition × age on the somatic area, but a significant effect on the dendritic area was associated only with nutrition (Table 2). A decrease in the somatic and dendritic areas in undernourished rats was observed, mainly at the ages of 12 ($P < 0.001$, $P < 0.002$) and 20 days ($P < 0.001$, $P < 0.01$) with no significant difference at 40 days (Fig. 2). The multivariate analysis showed no effects of the nutrition factor on the number of dendritic branches in the AM nucleus, but it depicted significant effects associated to both age and nutrition × age interaction (Table 3). There was only a significant effect of nutrition on the fourth-order dendritic branches, a significant effect of age on the first- and fourth-order dendritic branches and associated to the interaction nutrition × age on the first- and second-order branches (Table 3). No changes in the number of total dendritic branches were observed from Day 12 to Day 20 and to Day 40. At the ages of 12 and 20 days there was a statistical difference between the U- and the C-values, but on Day 40 no difference was observed (Fig. 3).

DISCUSSION

The undernutrition paradigm used for this study produced an important body and brain weight reduction. The body weight deficit oscillated in a range of 20 to 25%, whereas the brain weight deficit oscillated in a range of 5 to 8%. Even though the 40-day-old U-rats had been exposed to a nutritional rehabilitation for 16 days, the reduced body and brain weights remained. These data are similar to others referred to in previous studies with the same undernutrition paradigm (24) and with other undernutrition methods (16, 27). The less-intense effects of undernutrition on the brain than on body growth reflect the effects of the "brain sparing" mechanism, which has been previously discussed by other groups (5, 43, 45, 48).

The neurons sampled for each amygdaloid nucleus exhibited a significant reduction in somatic and dendritic area associated to neonatal undernutrition, especially at the ages of 12 and 20 days, but on Day 40 this difference disappeared (Fig. 2). In the C-group, the somatic area increased from Day 12 to 20, but suffered a reduction by Day 40. In the three nuclei the same phenomenon was observed and could be the expression of a possible maturational mechanism for size adjustment (28). In the U-group little or no modifications occurred in the somatic area of the three ages studied. Therefore, the minimal difference between both experimental groups at age 40 was possibly due to a lack or delay of the reduction mechanism in the U-group, observed in the C-group neurons.

The dendritic area, which reflects the extension and density of the dendritic tree, consistently revealed a reduction in the U-group at 12 and 20 days and in the three amygdaloid nuclei. In the C-group the main dendritic area values did not change from Day 20 to Day 40,

TABLE 3

ANOVA and MANOVA Results for the Number of Dendritic Branches in Three Amygdaloid Nuclei in the Rat

Dendritic order	ABL		ACE		AM	
	$F(1,44)$	$P<$	$F(1,44)$	$P<$	$F(1,44)$	$P<$
	Effects associated with the nutrition factor					
1st	5.54	0.02	4.02	0.02	1.95	NS
2nd	16.00	0.0001	1.06	NS	0.43	NS
3rd	7.20	0.008	4.70	0.03	0.63	NS
4th	7.65	0.006	5.69	0.01	4.30	0.04
5th	1.02	NS	0.82	NS	0.30	NS
6th	2.21	NS	0.20	NS	—	—
Total	3.3	0.003	2.15	0.04	1.45	NS
MANOVA	$df(7, 138)$		$df(7, 138)$		$df(6, 139)$	
	$F(2,44)$	$P<$	$F(2,44)$	$P<$	$F(2,44)$	$P<$
	Effects associated with the age factor					
1st	4.84	0.009	7.25	0.001	4.21	0.01
2nd	4.37	0.01	13.78	0.0001	0.84	NS
3rd	2.25	NS	19.33	0.0001	0.35	NS
4th	2.08	NS	11.89	0.0001	3.00	0.05
5th	4.23	0.01	5.30	0.006	0.67	NS
6th	5.61	0.001	1.42	NS	—	—
Total	2.64	0.001	4.73	0.0001	2.16	0.01
MANOVA	$df(14, 276)$		$df(14, 276)$		$df(12, 278)$	
	$F(2,44)$	$P<$	$F(2,44)$	$P<$	$F(2,44)$	$P<$
	Effects associated with the nutrition × age factor					
1st	0.34	NS	7.27	0.001	6.84	0.001
2nd	0.57	NS	0.18	NS	5.28	0.006
3rd	0.15	NS	0.75	NS	2.53	NS
4th	4.21	0.01	1.23	NS	2.12	NS
5th	0.58	NS	0.78	NS	0.98	NS
6th	2.30	NS	0.20	NS	—	—
Total	1.82	0.03	1.35	NS	2.35	0.007
MANOVA	$df(14, 276)$		$df(14, 276)$		$df(12, 278)$	

Note. NS, nonsignificant difference.

in contrast with the U-group which showed a continued growth to Day 40 probably associated with a plastic compensation mechanism. Therefore at the age of 40 days, following 16 days of nutritional rehabilitation, the dendritic area of the U-group reached control values.

By contrast, neonatal undernutrition affected the dendritic ramifications of the three amygdaloid nuclei to different degrees. Due to undernutrition the ABL and ACE nuclei demonstrated a significant decrease in the number of dendritic branches, which lasted up to Day 40. In the ABL, the ANOVA analysis indicated a prominent reduction associated with undernutrition in all dendritic orders, excepting the fifth-order branches, and in both experimental groups an increase from one age to the other was evident. Likewise the ACE demonstrated an intense effect on the first-, third-, and fourth-order dendritic branches, and a growth process of its dendritic tree could also be inferred. On the other hand, the AM suffered a minor impact on its dendritic tree, there was only a decrease in the fourth-order branches and no change in the total number of dendritic ramifications from one age to the other. Apparently, the ABL and ACE dendritic trees were more vulnerable to neonatal undernutrition than the AM nucleus.

The phase of rapid cell proliferation in the amygdaloid complex occurs between Gestational Days 12 to 17 (17). Then, the cells already existing grow to a larger size and undergo an important phase of dendritic ramifications, establishing functional connections with other neurons. Present findings suggest that the latter phase takes place in some amygdaloid nuclei during the postnatal period and may be vulnerable to perinatal insults, since at ages 12 to 40 days this process showed an important decrement associated with neonatal undernu-

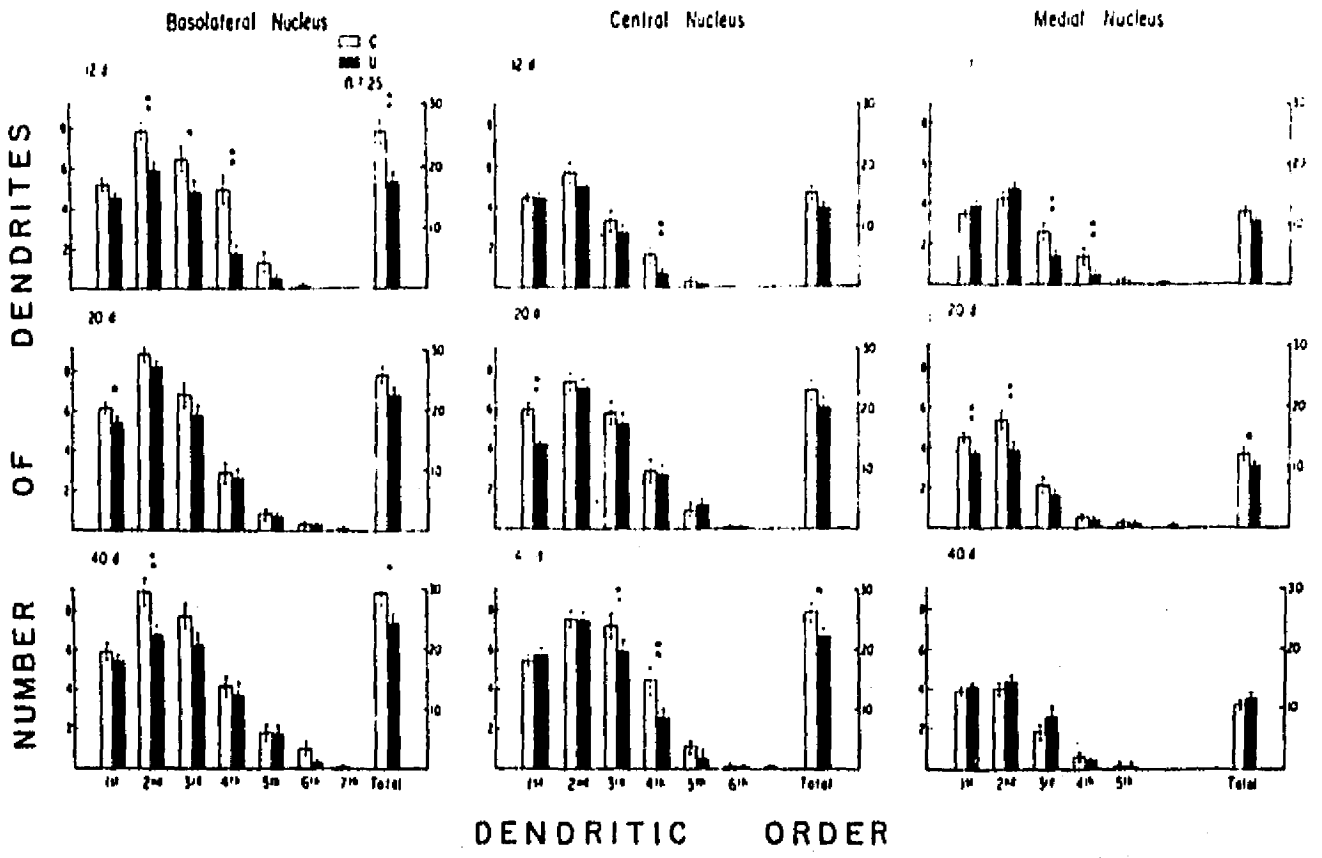

FIG. 3. Dendritic branches, mean values ± SEM in three amygdaloid nuclei at three ages of development in control and undernourished rats. $^{**}P < 0.001$, $^{*}P < 0.05$.

trition. Due to its early ontogenetical origin the AM might be less vulnerable to a postnatal insult than the ABL and the ACE nuclei, which undergo a late development process regarding their functional maturation. This assumption is supported in part by Kozik and Szczech (32) who described how the histochemical and synaptic maturation of the amygdaloid system was completed between ages 17 and 40 days, reaching peak values for the AM in an early stage of postnatal life and for the ABL around the 40th day.

It has been proposed (40) that multisensorial modulatory mechanisms are accomplished in late developmental phases and consequently may be altered by neonatal undernutrition, due to the nutritional supply limitation to the cell during the critical growth period and to a delay in the development of the sensory systems upon which multimodal sensory structures depend for their functional structuring. Hence, deficient nutritional supply and afferent input can lead to underdevelopment of the dendritic trees in modulatory integrative structures. The results of this study could reflect this phenomenon, since the ABL and ACE exhibited intense alterations associated with neonatal undernutrition. Both nuclei receive multimodal sensory afferents (12, 31, 33, 42, 46, 47, 58, 62) and have a relevant integrative and modulatory function for emotional motor and visceral responses (20, 29, 36, 37). In contrast, the AM is a specific relay zone for olfactory afferents associated with hormonal and sexual responses (38) and suffered a lower impact in its dendritic ramification due to neonatal undernutrition.

The findings reported in a previous study by Salas *et al.* (55) could support this interpretation. They described a reduction of somatic area, dendritic field, and dendritic length in the thalamic reticular nucleus of the rat, but did not find significant differences in the thalamic lateral nucleus. It is known that the reticular nucleus receives multisensorial afferents and exerts a modulatory influence on all thalamic nuclei inhibiting some sensory afferents (56). In contrast, the lateral nucleus is a specific structure which participates in a neural circuit for coordination of posture and muscular tone.

As described above, the amygdaloid complex plays an important role in integrating multimodal sensory information and elaborating emotional motor and visceral responses. A functional limitation in the amygdala due to deficient incoming information through a reduced dendritic tree may constitute the morphological basis for the characteristic emotional behavior observed in the undernourished rat.

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Dendritic Arbor Alterations in the Medial Superior Olivary Neurons of Neonatally Underfed Rats

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Key Words
Development
Superior olive
Neonatal undernutrition
Rat

Abstract
Golgi-Cox-stained bipolar cells of the medial superior olive (MSO) were analyzed in control and undernourished Wistar strain rats at 12, 20, 30 and 40 days of age. Undernutrition significantly reduced the number of dendrites and the extension of ipsilateral dendritic prolongations, with no effects upon the cross-sectional somal area and minimal alterations in the corresponding contralateral dendritic branches. The data suggest that in underfed rats, afferents from the receptors projecting to the MSO via the anteroventral cochlear nuclei may cause an imbalance in the binaural interactions which occur between the axon terminals and the ipsilateral and contralateral dendritic arbors of MSO neurons.

Introduction

The superior olivary complex is a group of brainstem nuclei closely related to the tuning of gating mechanisms by which sensory cues are modulated to permit habituation, attentive behavior [Oatman, 1976; Feng and Rogowski, 1980] and sound location [Erulkar, 1972]. Part of this complex is represented by the medial superior olive (MSO), predominantly constituted by bipolar cells with horizontally projecting dendritic arbors [Warr, 1966; Schwartz, 1977]. These dendritic trees have segments which receive input from both ipsilateral and contralateral anteroventral cochlear nuclei [Stotler, 1953; Harrison and Warr, 1962; Harrison and Feldman, 1970; Lindsey, 1975; White and Warr, 1983]. Moreover, the MSO is tonotopically organized, with high ventrally and low dorsally distributed frequencies [Warr, 1966]. It has extensive connections with the ipsilateral inferior colliculus [Stotler, 1953; Schwartz, 1977], receives binaural innervation [Warr, 1966; Yin and Chan, 1990] and contains cells that exhibit either binaural summation or binaural suppression responses [Warr, 1966; Yin and Chan, 1990]. Thus, the conspicuous location of the MSO neurons and their systematic pattern of innervation makes this nucleus a useful model to assess the effects of perinatal influences which affect hearing maturation.

Several procedures of perinatal experimental undernutrition in rats have revealed a significant reduction in body and brain weight, as well as in physical growth. Reduction in body size, sparse hair growth, and a 2- to 3-day delay in ear and eye opening [Mourek et al. 1967] are also consistently correlated with the delayed capacity to generate electrical brain activity in response to sensory stimuli [Callison and Spencer, 1968; Salas and Cintra, 1973].

The present study was undertaken to investigate the effects of neonatal undernutrition upon the morphology of the bipolar MSO neurons. Data indicate that early food

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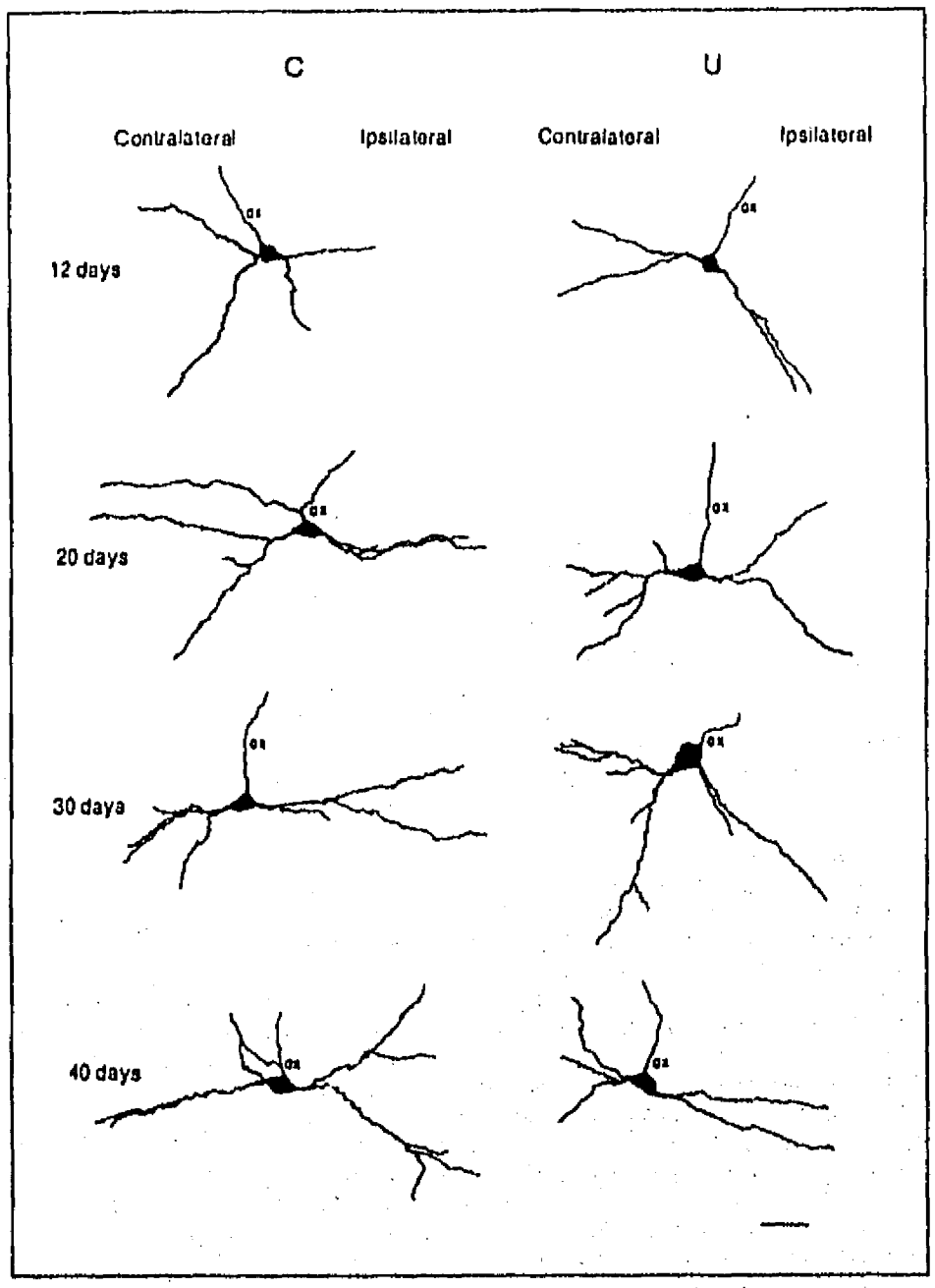

Fig. 1. Camera lucida tracings of bipolar MSO neurons from control (C) and undernourished (U) rats at 12, 20, 30 and 40 days of age, showing the ipsilateral and contralateral dendritic arbors. ax = Axon. Bar = 50 µm.

deprivation mainly impairs the development of the ipsilateral dendritic arbor, with minimal effects upon contralateral dendritic tree prolongations. The findings may be relevant to present knowledge on the generation of both binaural summation and binaural suppression responses at the supraolivary level and the possible modification of these functions in neonatally underfed rats.

Material and Methods

Animals

Fifteen timed pregnant Wistar strain rats *(Rattus norvegicus)* were housed individually in Plexiglas maternity cages (50×40×20 cm) with food (Purina chow) and water ad libitum in a temperature-controlled room (24 ± 1 °C) and a 10-hour dark, 14-hour light cycle (lights on at 7.00 h). At birth (day 0) animals were grouped into litters of 8 male pups per mother by adding or removing extra male pups. Neo-

Table 1. ANOVA for body and brain weight values of control and undernourished rats during development

Factor	d.f.	Body weight F	Brain weight F
Nutrition	1, 24	132.92*	57.60*
Age	3, 24	813.27*	104.44*
Nutrition × age	3, 24	10.56*	4.26**

*$p < 0.0001$; **$p < 0.01$. Each calculation typically reflects a total of n = 16 control and n = 16 undernourished rats.

natal undernutrition was produced by transferring daily the same half of the litter (n = 4) from the nest to an incubator maintained at 29°C, for 12 h (8.00–20.00 h), from postnatal days 1 to 24. In all cases, underfed pups were placed together inside the incubator to secure some physical interaction during the 12-hour period of environmental isolation, although with significant reduction in the external environmental sensory signals. The control group consisted of pups raised in undisturbed litters adjusted at birth to 8 pups per mother. Animals were sacrificed in groups for histological examination at 12, 20, 30 and 40 days of age. Individuals of the older groups were weaned at 25 days of age, after which they were subjected to a nutritional rehabilitation period with free access to water and food (Purina chow).

Histological Procedure

Eight subjects per group (4 controls and 4 experimental animals) were weighed and deeply anesthetized with ether. They were then intracardially perfused with saline and 10% neutral buffered formalin. After 12 h of fixation, the spinal cord was severed at the upper end of the cervical segment C1, and the entire brain was removed and weighed. Coronal sections (100–150 µm) from the medulla oblongata were stained according to the Golgi-Cox procedure. Location and identification of neural structures were based on the atlas of Sherwood and Timiras [1970]. The nomenclature and general location of the superior olivary complex was adopted from the atlas of Paxinos and Watson [1986]. Anterior-posterior coordinates for localization of the superior olivary complex corresponded to values from P 0.4 mm to P 1.4 from the younger to the older groups. Slides were coded to ensure that all observations were blind with respect to age and dietary treatment. Camera lucida drawings of each neuron were obtained at a magnification of ×675.

Morphometric Analysis

Twenty impregnated neurons (fig. 1), whose dendritic field appeared to be confined to one section as evidenced by light microscopy, were selected for each experimental condition, age group and neuronal parameter. From camera lucida drawings, the cross-sectional cell body area was measured with a Ladd graphical digitizer. Ipsilateral and contralateral dendritic arbor measurements were obtained by counting the number of 1st, 2nd, 3rd, 4th, 5th, 6th and the total neuronal dendritic orders. The extent of dendritic branching was analyzed by placing the border located between the cell body and primary dendrites at the center of the first ring of a series of 6 concentric rings (spaced at 40.4-µm intervals) and counting all intersections of dendrites with individual rings of each side of the neuron [Sholl, 1956]. No attempt was made to correct the compression of the three-dimensional dendritic tree to a two-dimensional sketch, since the relative differences between neurons remain constant when transformed from three to two dimensions [Spinelli et al., 1980].

Statistics

Experimental data were analyzed by using the Systat Statistical Package version 2.1. To examine the score differences of the different measurements among ages and dietary treatments, the following separate statistical analyses were used: (1) the values for body and brain weight were analyzed with a two-way ANOVA 2 (nutritional regimes) ×4 (ages); (2) cross-sectional cell body area scores (20 cells per age and nutritional condition) were compared in a two-way Anova 2 (nutritional regimes) × 4 (ages); (3) the impact of neonatal undernutrition upon the number and extension of each ipsilateral and contralateral dendritic branch was compared with a MANOVA 2 (nutritional regimes) ×4 (ages) ×6 (dendritic branches); (4) factors with a higher variance content were calculated with a canonical correlation analysis; (5) in all cases, the dendritic score differences between experimental groups for particular ages were calculated with the Newman-Keuls post hoc test. Each subject was treated as an independent sample in these analyses. The litter from which the subject came was ignored. The assumptions of independence of each neuronal parameter within the subjects and of the absence of correlation among littermates were not strictly justified. However, in spite of the complexity of the variables here evaluated and of the difficulty in ascertaining the lack of correlation, we believe that the data derived from statistical analyses give support to our conclusions.

Results

Body and brain weight comparisons among groups at each developmental age showed significantly lower values for neonatally underfed rats than for controls (table 1). Analysis of the mean cross-sectional cell body area throughout the experimental period indicated that bipolar MSO neurons were not affected by neonatal undernutrition, although this parameter was significantly affected ($p < 0.01$) when age was considered (table 2).

Our undernourished rats showed a 2- to 3-day delay in ear and eye opening compared to controls. Light-microscopic observations from the present material revealed that MSO neurons were spindle-shaped with dendritic arbors directed in a perpendicular direction to the perikaryon (fig. 1). They usually exhibited two 1st-order dendrites from which dendrites of 2nd, 3rd, 4th, 5th and 6th orders ran to the edges of the nucleus at an angle of 40° or less with respect to the cell plate, as described elsewhere [Schwartz, 1977].

The total number of ipsilateral dendritic branches was significantly reduced in neonatally underfed rats ($p < 0.001$), with differences particularly evident in the 3rd and 4th dendritic orders (table 2). Age did not modify the

Table 2. Neuronal measurements in MSO bipolar cells of control and undernourished rats at 12, 20, 30 and 40 days of age: F values

Neuronal measurement	Nutritional regimes (d.f. 1, 152)		Age (d.f. 3, 152)		Interaction (d.f. 3, 152)	
	ipsilateral	contralateral	ipsilateral	contralateral	ipsilateral	contralateral
Cross-sectional cell body[a] area	1.135		3.847**		1.765	
Dendritic order[b]						
1	1.011	0.129	0.944	0.386	0.832	0.386
2	0.241	2.928	0.241	3.230*	0.241	3.197*
3	61.671***	3.201	2.683*	3.424*	2.163	0.062
4	26.395***	0.008	0.290	0.910	0.432	0.888
5	0.679	1.286	1.131	0.524	0.226	0.524
6	0.200	0.333	0.733	1.222	0.200	0.333
Total	52.394***	1.724	2.080	3.226*	0.831	0.280
Dendritic intersections[b]						
1	1.989	0.193	1.430	0.623	0.843	1.453
2	13.703***	1.157	1.217	1.424	0.527	0.090
3	28.570***	1.169	0.465	1.303	4.669**	1.181
4	43.996***	0.790	0.991	0.376	1.499	0.431
5	20.411***	3.705*	0.417	4.414**	0.145	1.431
6	7.092**	2.696	0.613	4.014**	0.496	0.85
Total	60.757***	5.780**	0.743	4.711**	2.471	0.933

*p < 0.05; **p < 0.01; ***p < 0.001.

[a] Statistical comparisons of n = 20 samples per age and condition.

[b] Each calculation typically reflects n = 20 samples per age and condition.

number of branches of most ipsilateral dendritic orders (table 2) although a significant reduction in the 3rd ipsilateral dendritic order was observed (table 2). No significant interaction was observed in the number of ipsilateral dendritic orders between dietary treatment and age. In contrast, neonatal undernutrition did not cause significant differences in the number of contralateral dendritic orders (table 2, fig. 2), although significant age-related changes on proximal dendrites (2nd and 3rd contralateral dendritic orders, not shown in fig. 2) were evident (table 2). Moreover, no significant interaction was observed between the nutritional and age factors with respect to measurements of most ipsilateral and contralateral dendritic branches (table 2).

In neonatally underfed rats, the total number of ipsilateral dendritic intersections showed a significant reduction ($p < 0.01$), with main effects from the 2nd (80.8 μm) to the 6th (242.4 μm) circle (table 2). The age factor did not affect the number of ipsilateral dendritic intersections (table 2), although a significant interaction between the nutritional and age factors was observed in the 3rd (121.2 μm) circle. Total contralateral dendritic intersections were affected by food deprivation ($p < 0.01$; table 2, fig. 3), with significant effects only in the 5th (202 μm) circle (table 2). Total contralateral dendritic intersections were also affected by age ($p < 0.01$), with significant effects for the 5th (202 μm) and 6th (242.4 μm) circles (table 2, fig. 3). Moreover, no significant interaction between the nutritional and age factors was found in contralateral dendritic intersections (table 2).

The canonical discriminant analysis of ipsilateral dendritic branches resulted in significant differences among nutritional regimes ($p < 0.0001$) and helped to illustrate that the 3rd and 4th dendritic orders, which had a standardized coefficient greater than 0.5, contributed most in the underfed group to ipsilateral dendritic arbor differences among groups (table 3). Moreover, the canonical discriminant analysis of ipsilateral dendritic intersections showed significant differences between dietary treatments ($p < 0.0001$) and indicated that the 3rd, 4th and 5th dendritic intersections with coefficients greater than 0.5 contributed most in the underfed group to the significant differences for this neuronal parameter (table 3).

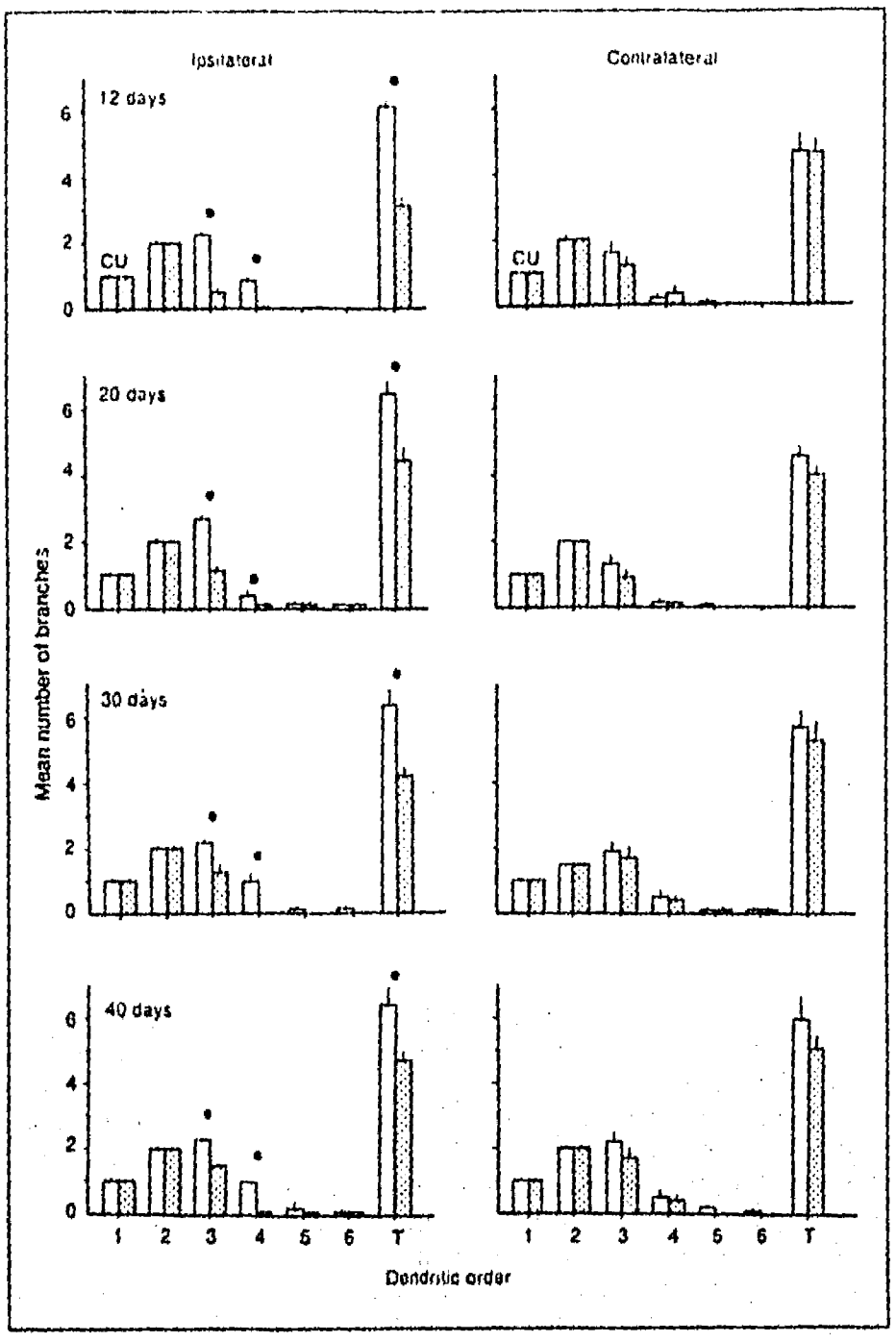

Fig. 2. Number of ipsilateral and contralateral dendritic branches in control (C) and neonatally underfed (U) rats during development. Undernutrition significantly reduced the number of ipsilateral 3rd and 4th dendritic orders as well as the total number of dendrites (T), with no effect on contralateral dendritic orders. Black dots above bars indicate significant differences. Figures on the upper left side indicate different developmental ages. Data indicate the average of 20 cells per group and nutritional condition.

Discussion

The present data show that neonatal undernutrition differentially affects the growth of the dendritic arbors of bipolar MSO neurons. This effect is characterized by a significant reduction in the number of dendritic branches, of ipsilateral dendritic length, and of dendritic growth, with minimal significant effects upon the contralateral counterpart.

The finding of a differential effect between the ipsilateral and contralateral MSO dendritic arbors may be explained by the severe effect of neonatal undernutrition upon the anteroventral cochlear nuclei and/or the maturation of the outer hair cells. This effect continues through the first 2 postnatal weeks [Pujol et al, 1978; Kelly, 1985], presumably reducing the anteroventral cochlear afferent discharges upon the ipsilateral dendritic arbor of neurons of

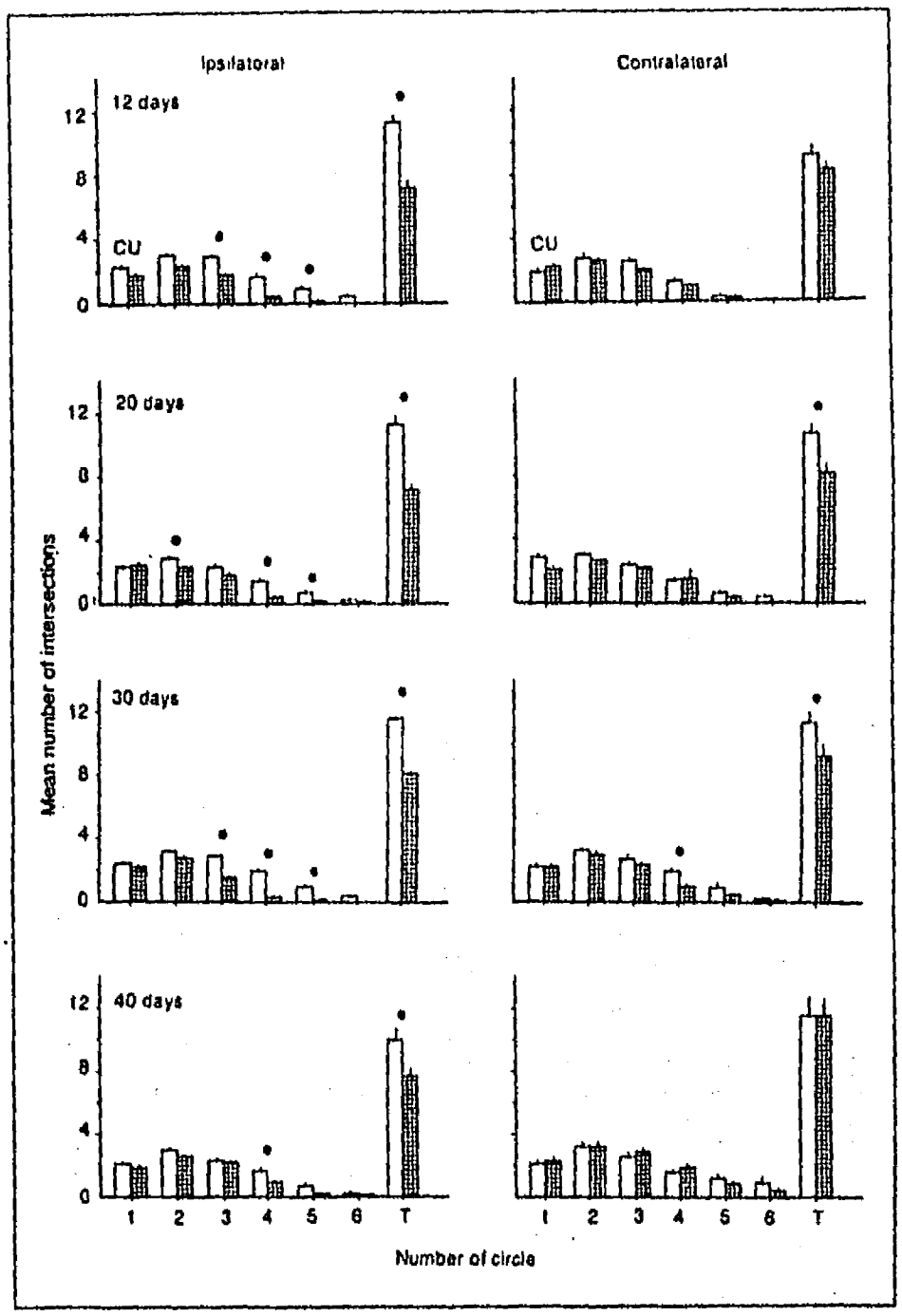

Fig. 3. Number of ipsilateral and contralateral dendritic intersections in control (C) and neonatally undernourished (U) rats during development. Undernutrition significantly reduced the number of intersections from the 2nd (80.8 μm) to the 5th circles (202 μm) at 12–30 days of age and in the 4th (161.6 μm) circle at 40 days of age, as well as for total (T) intersections. Undernutrition provoked minimal contralateral effects. Symbols, number of cells and codes as in figure 2.

the MSO and so producing the morphological effects reported here. This assumption is supported by previous evidence showing that one lesion of the anteroventral cochlear nucleus results in shrinking of the partially deafferented MSO cell dendrites, without alterations in the opposite dendritic growth [Liu and Liu, 1971]. The undernourishing procedure used here was associated with different stressful situations caused by the daily 12-hour period of sensory deprivation due to the separation of the pups from their mother, littermates and the nest, and their isolation in the incubator. This situation may resemble the symmetrical sound deprivation of neonatal rats binaurally deprived by the ligation of the external auditory meatuses where no effects on binaural interaction have been described [Silverman and Clopton, 1977]. However, if neonatal undernutrition and its associated influences interfere

Table 3. Canonical loading correlations between conditional dependent variables and dependent canonical factors: individual orders and dendritic intersections with standardized coefficients

Canonical factors	Standardized coefficients
Cross-sectional somatic area	0.113
Dendritic orders	
Ipsilateral	
1st	0.110
2nd	−0.054
3rd	0.836[1]
4th	0.569[1]
5th	0.091
6th	0.049
Contralateral	
1st	0.040
2nd	−0.180
3rd	0.193
4th	−0.010
5th	0.125
6th	0.063
Dendritic intersections	
Circle	
1	0.158
2	0.417
3	0.583[1]
4	0.742[1]
5	0.515[1]
6	0.302
Circle	
1	0.049
2	0.121
3	0.121
4	0.099
5	0.211
6	0.180

[1] Denotes dependent variables that have high standardized coefficients.

more with the maturation of the outer hair cells and their connections during the first 2 postnatal weeks than with the precocious connectivity of inner hair cells already present at birth [Pujol et al., 1978], this may result in a different timing of comparative converging influences upon the ipsilateral and contralateral MSO dendritic arbors affecting the development of brainstem auditory binaural interactions.

The morphological observations of the present study suggest that underfed rats may reveal an impairment in the auditory integrative mechanisms underlying possibly MSO-altered binaural interactions. The data suggest that efferent discharges arising from the anteroventral cochlear nuclei may unbalance the interactions between axon terminals and ipsilateral and contralateral dendritic arbors of bipolar MSO neurons. This inbalance may cause alterations in the MSO efferent discharge, interfering with the binaural interactions at supraolivary levels [Inbody and Feng, 1981; Yin and Chan, 1990]. MSO neural activity is one of the first steps of the binaural interaction processes necessary for normal auditory perception [Kelly, 1985], but it may not be the only one. Binaural interactions can occur independently of the participation of the superior olive, and binaural responses in the inferior colliculus of the rat are maintained following kainic acid lesions of MSO nuclei [Li and Kelly, 1992]. The present data may also help explain the impairment in the modulation of the startle response to a loud and sharp noise associated with increased plasmatic corticoid levels [Melia et al., 1992] and enhanced emotionality seen in neonatally underfed adult rats [Levitsky and Barnes, 1970], and in the habituation phenomenon provoked by repetitive auditory stimulation in neonatally underfed children [Lester et al., 1975]. Moreover, they are consistent with the alterations in thalamic neurons subserving selective attention to environmental cues [Salas et al., 1986]. This assumption is supported by electrophysiological and morphological data in olfactory and visual channels of neonatally undernourished rats [Salas et al., 1977; Math and Davrainville, 1980], suggesting an impairment in the sensorial integrating mechanisms underlying selective attention and perceptual phenomena.

Acknowledgments

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Dendritic Branching of Claustral Neurons in Neonatally Undernourished Rats

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Key Words
Undernutrition, rats
Dendritic development
Claustrum

Abstract
Golgi-Cox-impregnated neurons of the claustrum were studied in control and undernourished Wistar strain rats at 12, 20, and 40 days of age. A reduced cross-sectional somatic area was observed in the 20-day-old undernourished rats and a significant reduction in the dendritic area was observed in the three ages studied. Dendritic arbor alteration was mainly observed in the number of high order and in the total number of dendritic branches of undernourished rats throughout the study. The data suggest vulnerability of the dendritic claustral growth process during the postnatal period to neonatal undernutrition. These alterations may be associated with telencephalic integrative impairments described in perinatal undernourished rats.

Introduction

During development, brain structures undergo a progressive heterochronic growth to achieve a complex morphofunctional substrate and confront environmental demands. Thus, some of the sensorial systems are quite advanced in their development at birth and reach maturity at an early ontogenetic age. Nevertheless, multisynaptic modulator brain structures attain maturation and adjust their functional connections late in brain ontogeny [1] in order to moderate the sensory ascending information. Morphological evidence has revealed that during neurogenesis interneurons as fundamental substrate of multisynaptic modulator pathways arise late prenatally and their proliferation and functional organization continues throughout the postnatal period [2, 3]. These interneurons participate in

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complex physiological phenomena such as sensory integrative mechanisms, learning and mnemonic processes [4]. Consequently, perinatal insults such as abnormal hormonal levels, drugs, undernutrition and neonatal handling interfere with developmentally delayed multisynaptic modulator systems [5–10].

The claustrum has been recognized as a polysensory telencephalic structure constituted by a group of displaced cortical interneurons [11, 12] that establish extensive reciprocal connections with the visual, motor, auditory, enthorrinal, orbital, prepyriform and cingular limbic cortices [13–17] and with the use of autoradiographic techniques it has been demonstrated that afferent information is arranged in a topographical order [15, 16]. Also, the main cortical input to the claustrum arises from pyramidal cells from layer IV and the return pathway arrives at all cortical layers, but mainly at layers IV and VI [18].

Several studies suggest that the claustrum plays an important role in coordinating visual input with gross motor control and movement detection [12, 19]. Also, due to its extensive reciprocal projections with the neocortex, it has been considered an integrative polysensory structure [13, 16] that may play an important role in motivational and mnemonic processes [17] and it also provides an important substrate for interhemispheric communication [20].

Because of its extensive corticocortical sensory connections, the claustrum may be an interesting structure to assess the alterations provoked by different perinatal insults on the sensory integrative maturational processes. In order to observe somatic and dendritic characteristics of the claustrum, undernourished and control rat brains at 12, 20 and 40 days of age were prepared with the Golgi-Cox staining procedure and cross-sectional somatic and dendritic neuronal measurements were performed.

Method

Animals. For this study male Wistar strain rats were used. Three groups of 2 adult male and 5 female rats weighing 250–300 g were housed in transparent plastic cages (50 × 40 × 20 cm) for mating. They were maintained in an ad libitum diet of Purina chow pellets and water, and were kept at room temperature of 24°C and a 12:12-hour light-dark cycle (lights on at 07.00 h). Two days before delivery females were placed individually in acrylic cages (45 × 30 × 20 cm), with clean shavings for nesting and free access to food and water.

On postnatal day 1, the litters were adjusted to 8 pups, 4 females (F) and 4 males (M) and their body weight was registered. One half of the pups in each litter, 2 F/2 M, was assigned to the undernourished group (U) and the other half served as control group (C). Although litters contained pups from both sexes, only male pups were considered for this study, in order to avoid a sex associated difference.

Undernourishing Procedure. Neonatal undernutrition was produced by daily removing the U pups from the nest and keeping them for 12 h in an incubator (08.00–20.00 h) maintained at 29°C. The other 12 h they were returned to the dam in order to enable them to suckle the nursing female. The U pups were subjected to this procedure throughout lactation (postnatal days 1–23). This procedure reduces the pups opportunities for suckling and may partially reduce the maternal-pup interactions. During this period the C pups remained in the nest undisturbed with free access to the dam. The undernourishing procedure was suspended at 24 days of age, because during the first 3 postnatal weeks the brain is extremely vulnerable to external influences [3]. From then on the U pups were exposed to nutritional rehabilitation and were, therefore, left to remain in their home cage as their C siblings with free access to the dam, food (Purina chow) and water. In order to recognize the U pups from their C siblings in the nest, their heads were marked with a dot of colored washable ink. Pups were weaned until day 28 and housed in groups of 6–8 rats of the same sex and nutritional treatment, since early weaning can lead to the litter's physical impairment, and in order to permit a better nutritional rehabilitation [21].

Surgical and Staining Procedure. On postnatal days 12, 20 and 40, male pups from each condition (n = 8) were weighed, anesthetized with ether and sacrificed by decapitation. Immediately their brains were removed and weighed, cut in four coronal blocks and immersed in a Golgi-Cox solution [22] for impregnation. After 3 weeks, the blocks were dehydrated and

Table 1. Effect of neonatal undernutrition on body and brain weight during development in rats

Age days	Body weight		Brain weight	
	C group	U group	C group	U group
12	19.85±0.95	14.90±1.04*	0.98±0.04	0.90±0.04
20	37.33±1.30	28.12±0.59**	1.46±0.02	1.38±0.02*
40	100.12±3.61	81.53±3.09*	1.73±0.03	1.60±0.02*

Factor	d.f.	Body weight		Brain weight	
		F	p<	F	p<
Nutrition	1,42	40.18	0.0001	12.33	0.001
Age	2,42	674.57	0.0001	249.63	0.0001
Nutr × age	2,42	5.46	0.008	0.30	NS

NS = Statistically nonsignificant.
* $p < 0.01$; ** $p < 0.001$ by Newman-Keuls test.

embedded in low viscosity nitrocellulose. Subsequently they were cut in coronal sections of 100–150 µm and mounted in serial order. For further identification slides were coded to ensure blind observations with respect to age and experimental condition. Location and identification of neural structures were based on the Sherwood and Timiras [23] atlas. Samples were taken from sections corresponding to the antero-posterior coordinates A 3.5–2.3 mm for 12-day-old brains, A5.0–3.5 mm for the 20-day-old group and A5.3–4.1 mm for the 40-day-old group. In all cases samples corresponded from the mid-anterior to the posterior portions of the claustrum.

Morphometric Analysis. Because the Golgi-Cox method specifically stains the neuron with its dendritic arbors with respect to length, number and orientation, drawings of complete and well impregnated neurons (fig. 1) whose dendritic field appeared to be confined to the section were obtained with a camera lucida adjusted to a light microscope at 675 × magnification. Five brains of each age and experimental condition were examined, and 5 neurons from each were obtained, resulting in a total of 150 neurons for the study. Cross-sectional cell body and dendritic areas were measured with a Ladd graphical digitizer and for this purpose, a line was traced connecting the tips of the peripheral dendrites. The drawings were placed on the digital surface and the contour of the resulting area and of the soma were retraced with a fine tip. The dendrites were classified into primary, secondary, tertiary order and so on, considering their place of origin and according to this strategy the number of dendrites of different orders was counted.

Statistics. Data obtained were analyzed with the Systat Statistical Package. For all experimental groups and parameters the interanimal variability was estimated with a one-way ANOVA. The values for body and brain weight, for cross-sectional somatic and dendritic areas and for every dendritic order were analyzed with a two-way ANOVA (nutrition × age). In addition, total values for all dendritic orders were analyzed with a MANOVA (nutrition × age × dendritic order) and a canonical correlation, which indicated the dendritic order most affected by undernutrition. The statistical difference between experimental groups for particular ages was calculated with the Newman-Keuls post hoc test..

Results

Significant diet (nutrition) effects were found for body weight in the three age groups studied and for brain weight in the 20- and 40-day-old groups (table 1). The reduction in body weight of the U pups reached a proportion of 25% at the age of 20 days and it diminished to 20% at day 40, while the deficit in

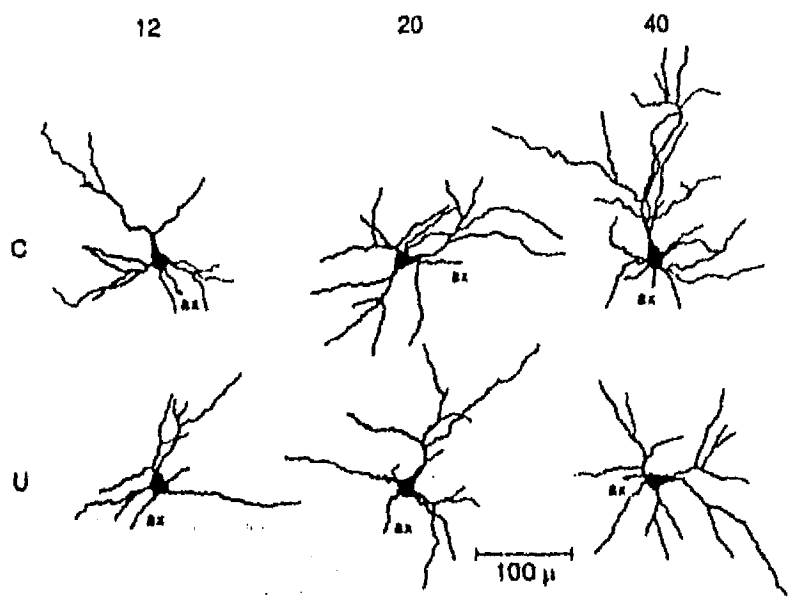


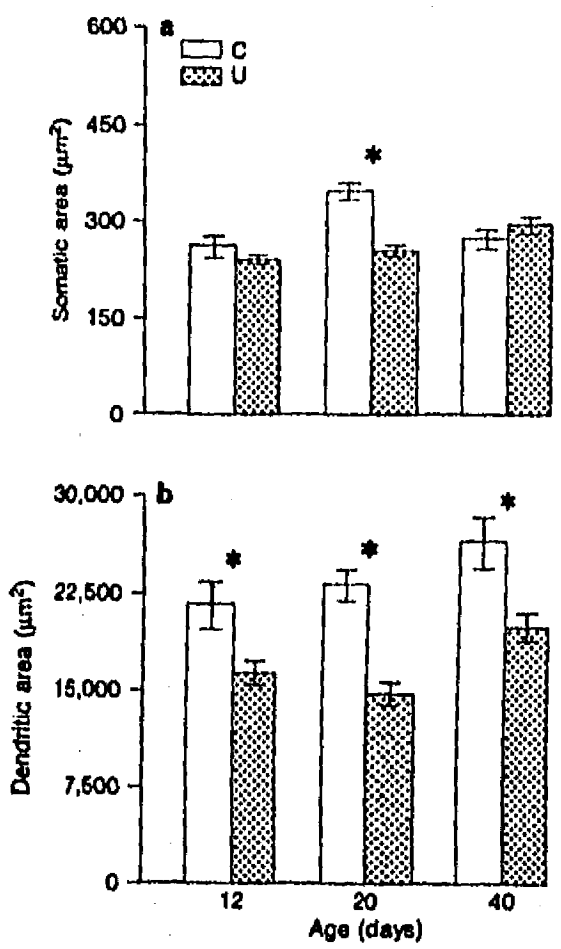
Fig. 1. Neurons of the claustrum drawn from U and C rat brains at 12, 20 and 40 days of age; ax = axon.

brain weight oscillated between 6 and 9% in the three ages studied. These findings are in agreement with those described in previous studies [24, 25] and they confirm the efficiency of the paradigm used to obtain undernourished pups.

Neurons observed in the claustrum were medium-sized with an irregular oval or pyramid-like soma giving origin to 3–4 primary ramified dendrites (fig. 1). The cross-sectional somatic area in the cells of the U group neurons was reduced on day 20, but not on day 12 and day 40 postnatally (fig. 2a). However, the ANOVA test indicated a relevant global influence of the nutrition factor on this parameter, $F(1,144)$ 5.44, $p < 0.02$, it also indicated an important influence of the age factor, $F(2,144)$ 4.77, $p < 0.01$; and of the nutrition × age factor, $F(2,144)$ 4.77, $p < 0.01$; and of the nutrition × age factor, $F(2,144)$ 6.22, $p < 0.003$. No interanimal variability was observed in this and other parameters.

On the other hand, the dendritic area in neurons of the U group was importantly reduced in the three ages studied (fig. 2b). The ANOVA test indicated a significant effect exerted by the nutrition factor $F(1,144)$ 35.28, $p < 0.001$; and the age factor $F(2,144)$ 6.66, $p < 0.002$, on this neuronal measurement. For the three ages studied the difference between groups was statistically significant, showing no recovery in spite of the nutritional rehabilitation at 40 days of age.

The number of dendritic branches also showed a significant reduction associated with the nutrition factor, Wilks' lambda = 0.872; $F(7,138)$ 2.906, $p < 0.007$; and with age factor, Wilks' lambda = 0.004, $F(14,276)$ 2.34, $p < 0.004$. In both experimental groups a growth process could be observed, since the amount of total dendritic branches increased from day 12 to day 40. Moreover, the number of dendrites in the claustrum of the U group exhibited in the three ages reduced values compared to the C group (fig. 3). This reduction in the U pup dendrites was more intense in the high order dendritic branches than in the primary, secondary or tertiary ramifications (ta-



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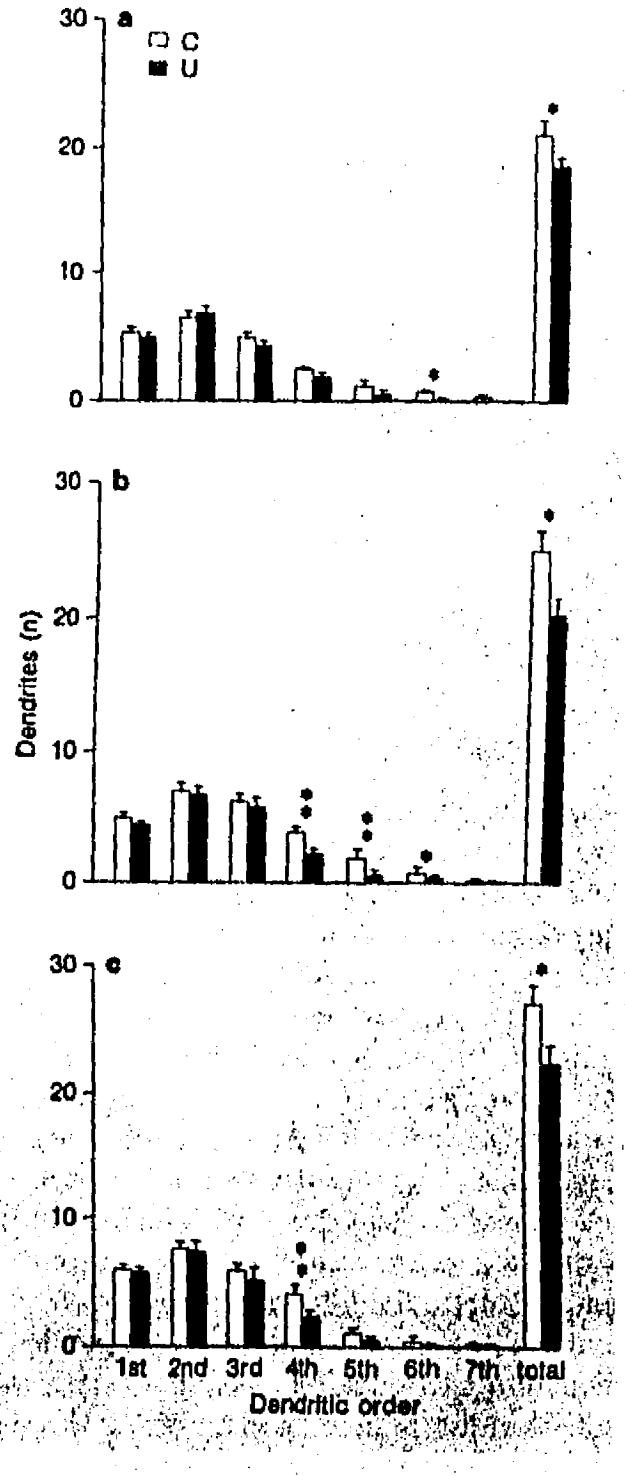

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Fig. 2. Mean values ± SEM for the cross-sectional somatic (**a**) and dendritic (**b**) areas of claustrum neurons in C and U rats (n = 25) at three developmental ages. * $p < 0.01$, statistically significant difference between U and C group for every age, obtained with Newman-Keuls test.

Fig. 3. Dendritic branching for claustrum neurons at three developmental ages (**a** 12 days, **b** 20 days. **c** 40 days) in neonatally U and C rats (n = 25); * $p < 0.05$; ** $p < 0.01$.

ble 2), as was indicated in the canonical correlation test. This tendency was already present at the age of 12 days, it became more evident on day 20, and remained on day 40 in spite of 16 days of nutritional rehabilitation (fig. 3).

Discussion

Present data show that postnatal undernutrition affects the dendritic outgrowth in the claustrum since its dendritic extension and

Table 2. Multivariate values indicating statistical significance for effects associated to undernutrition on dendritic branches, and canonical correlation indicating the most affected dendritic orders due to undernutrition

Dendritic order	F value d.f. (1,144)	p value	Canonical correlation
1st	2.57	NS	0.34
2nd	0.04	NS	0.04
3rd	1.66	NS	0.28
4th	14.56	<0.0001	0.82[1]
5th	9.20	<0.003	0.65[1]
6th	7.62	<0.007	0.59[1]
7th	2.01	NS	0.30

NS = Nonsignificant.
[1] Dendritic orders most affected by undernutrition.

dendritic branching suffered a significant reduction due to neonatal undernutrition and the alteration remained in spite of 16 days of nutritional rehabilitation. Our data point out that the extension and number of dendritic branches increased from day 12 to day 20 and to day 40 and consequently we can propose that this neuronal process undergoes an important early postnatal growth period. We observed that claustrum dendritic arbors develop in a centrifugal fashion with higher order branches being successively added throughout early postnatal life. Moreover, it was evident that this growth process is vulnerable to neonatal undernutrition, since the extension and dendritic branching exhibited a slower growth rate associated with this insult in the three ages studied and in spite of 16 days of nutritional rehabilitation for the day 40 group. The findings from Iñiguez et al. [26] support this observation, since they describe a substantial development, in the amount of afferent and efferent connections in the claustrum from birth and until postnatal day 14.

Morphological studies have shown that dendritic arbor space represents a preferential territory for specific afferent systems, and that peripheral dendritic branches are the most plastic components highly dependent on sensory information [27]. Herefore, the reduced high order dendritic branching of claustral neurons observed in undernourished rats may restrict their capability to receive and integrate afferent inputs arising from the cerebral cortex, and to organize the efferent discharges toward cortical and subcortical structures. This assumption is supported by multiple descriptions showing the high dependence of the claustrum activity on cortical inputs [13, 15, 17]. In this study the neuronal samples were taken mainly from the mid-anterior to the posterior portions of the claustrum which establish a reciprocal interaction with the motor, somatosensory, auditory and visual cortices [16, 20]. Therefore, reduced dendritic ramifications may be reflecting the effects of perinatal undernutrition as well a deficient afferent input arising from these cortical zones associated to early sensory deprivation.

Polysensory relay structures establish attentional states to specific information and maintain a certain excitability of the neural processes [3]. The claustrum, due to its strategic position and polymodal sensory inputs through its projections, may regulate attentional states and the integration of motor and sensory information. In this regard, projections from the visual cortex to the claustrum originating in the fourth layer and returning from the claustrum to the same cortical areas to layers IV and VI establishing a reciprocal modulator circuit, which may regulate sensory influx to this zone [18]. In undernourished rats the reduced dendritic arbor in the claustral neurons may generate a deficient regulatory process that may be associated with inadequate locomotion [28, 29] or with impaired visuospatial integration as observed in be-

havioral studies [30–32]. This possibility is strengthened by data showing the high vulnerability of polysensory nervous structures to perinatal undernutrition in the thalamus [1, 9], the amygdala [5], the hippocampus [3, 33, 34], the cerebellar granular layer [35] and the interneurons of the cerebral cortex [36]. However, further morphological and behavioral studies will be needed in order to corroborate this proposal.

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