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INTEGRACION ANATOMICA Y RECUPERACION FUNCIONAL DE LOS TRANSPLANTES NEOCORTICALES DE TEJIDO CEREBRAL FETAL

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PRESENTACION

En la serie de estudios que se muestran en el presente trabajo, hemos tenido la oportunidad de conjuntar dos importantes herramientas, los transplantes de tejido cerebral fetal y el modelo conductual del Condicionamiento Aversivo a los Sabores, que nos han permitido aproximarnos a una de las expresiones más apasionantes de la plasticidad del Sistema Nervioso de los seres vivos que es el aprendizaje.

El Condicionamiento Aversivo a los Sabores (CAS), constituye un modelo sólido para el estudio de la ontogenia y filogenia de los procesos gustativos. Además, ha contribuido a mejorar el entendimiento de los procesos anatómicos y/o funcionales del Sistema Nervioso Central (SNC), que integran la información gustativa. El modelo del CAS consiste brevemente, en la presentación de un estímulo gustativo (estímulo condicionado) seguido de una irritación gástrica (estímulo incondicionado) produciendo una aversión al estímulo gustativo, cuando nuevamente es presentado al sujeto experimental.

Los transplantes de tejido cerebral fetal constituyen un método de aproximación al conocimiento de los mecanismos inherentes a los orígenes, desarrollo y posibles soluciones de los trastornos del SNC, así como a la ontogenia del mismo.

En nuestro laboratorio se ha demostrado que transplantes homotópicos fetales de neocorteza gustativa (NG), producen recuperación en la capacidad de adquirir el CAS, después de dos meses, en ratas que previamente lo habían perdido debido a la lesión de la corteza. Posteriormente, tratando de conocer los mecanismos implicados en tal recuperación, mostramos que los transplantes homotópicos de NG pero no los heterotópicos de tejido tectal, podían restablecer las funciones cognitivas y la conectividad con el tálamo y la amígdala del tejido huésped, con

quienes la corteza gustativa mantiene conexiones normalmente.

Más tarde, con el ánimo de comprender los procesos temporales que subyacen a la recuperación funcional y anatómica observada, efectuamos un análisis conductual y citoarquitectónico, siguiendo el curso temporal (15, 30, 45 y 60 días) de desarrollo de los transplantes, mostrando que la recuperación conductual comienza a manifestarse a partir de los 30 días de desarrollo post-transplante, al tiempo que aparecen los primeros indicios de reconectividad, vascularización y maduréz estructural de nuestros transplantes, alcanzando su mejor expresión hacia los 60 días.

Estos resultados sugieren que la maduréz morfológica y la reconectividad entre el transplante y el huésped, son necesarias para la adecuada expresión de la recuperación conductual del CAS, en sujetos previamente lesionados en la neocorteza gustativa.

INTRODUCCION

LOS TRANSPLANTES DE TEJIDO NERVIOSO FETAL Y LA PLASTICIDAD DEL SNC

Hoy en día, se sabe que el Sistema Nervioso Central (SNC) es capáz de generar procesos que promueven la recuperación de funciones después de haber sido dañado. En respuesta a la denervación por ejemplo, numerosas fibras presentan rebrote axonal (sprouting) y forman nuevas sinapsis que reemplazan a las perdidas. En algunos casos, tales procesos de reconexión pueden participar en la recuperación funcional (Cotman *et al.*, 1981). Sin embargo en casos de daño severo, es necesario coadyuvar con los procesos naturales a fin de promover la recuperación funcional. Los transplantes de tejido cerebral fetal han sido ampliamente utilizados como coparticipes de los citados procesos de recuperación (Bjorklund y Stenevi, 1984; Gash *et al.*, 1985).

Los transplantes pueden actuar a varios niveles a fin de estimular la recuperación conductual. Por ejemplo, los transplantes pueden reconectar los circuitos neuronales que fueron interrumpidos tras una lesión; pueden asimismo incrementar la disponibilidad de un neurotransmisor para facilitar la comunicación neuronal. Los transplantes pueden también estimular la vascularización, eliminar substancias tóxicas o promover la sobrevivencia neuronal y el crecimiento a través de interacciones tróficas entre el huésped y el transplante (Cotman y Kesslak, 1988).

Las investigaciones en torno a la participación de los transplantes en los procesos de integración anatómica, han permitido el establecimiento de algunas premisas que hasta el momento parecen ser aplicables a la mayoría de los transplantes: a) Las células neuroepiteliales de las diversas regiones estudiadas continúan proliferando después de transplantarse al SNC produciendo neuronas con características morfológicas normales; b)

las células que se diferencian antes de que se efectúe el transplante, son capaces de sobrevivir y mantener el arreglo citoarquitectónico original en su nuevo ambiente (Kromer *et al.*, 1980).

Se ha definido asimismo una serie de variables que son fundamentales para realizar con éxito los transplantes de tejido nervioso; estas variables son: la edad del donador, el sitio del transplante y la edad del huésped. El transplantar tejido cerebral fetal al SNC adulto conlleva el siguiente juego de interacciones; 1) el SNC del huésped reacciona ante el transplante, 2) los transplantes fetales reaccionan ante la pérdida de su ambiente normal, aunque su crecimiento y diferenciación son restaurados posteriormente y 3) el transplante y el huésped tienden a establecer comunicación a través del intercambio de conexiones recíprocas. A este respecto, Gibs y Cotman (1987) sugieren en su trabajo sobre la sobrevivencia y crecimiento de los transplantes que: a) el introducir un lapso entre la lesión y el transplante incrementa la probabilidad de sobrevivencia y un adecuado desarrollo de los implantes y b) que generalmente las fibras formadas en los transplantes compiten selectivamente con las proyecciones nativas del huésped durante el proceso de reinervación.

LA INTEGRACION NEUROANATOMICA DE LOS TRANSPLANTES Y LA RECONECTIVIDAD

El advenimiento de la técnica de los transplantes cerebrales, ha permitido una aproximación al estudio del desarrollo y regeneración de conexiones neuronales en el SNC de mamíferos (Bjorklund y Stenevi, 1984).

Durante la década pasada se han registrado grandes avances en este campo, respaldando así, los primeros indicios positivos que sobre implantes de tejido nervioso se habían obtenido hacia principios de siglo. Hoy en día, se sabe que prácticamente todas las secciones del neuroeje pueden ser transplantadas con grandes

probabilidades de sobrevivencia, no solamente en SNC de animales neonatos o jóvenes sino también en el cerebro y la médula espinal de individuos adultos.

Los estudios clásicos sobre los mecanismos de regeneración neuronal en vertebrados inferiores, han proporcionado las premisas básicas para las nuevas investigaciones al respecto. Matthey (1926), Stone (1944), Stone y Zaur (1940) y Sperry (1947), mostraron que algunas estructuras oculares transplantadas o reimplantadas en salamandras adultas, eran capaces de regenerar nuevamente los patrones de conexión retinotectal, restaurando por ende la visión. Otros ejemplos de transplantes funcionalmente exitosos en vertebrados inferiores, son los implantes de segmentos de médula espinal en anfibios y aves jóvenes, los cuales, establecen conexiones neuromusculares que permiten así, el movimiento normal y coordinado de las extremidades (Detwiler, 1936; Piatt, 1940; Székely, 1963). Por su parte las investigaciones efectuadas en invertebrados, también han jugado un papel importante en el estudio de la regeneración neuronal debida a los transplantes. Así en 1986, Fredman y Gage efectuaron una serie de investigaciones que aportan sólidas evidencias para la regeneración de conexiones sinápticas específicas, en el molusco Aplysia. De esta manera, queda de manifiesto que entre las características más relevantes de los transplantes neuronales intracerebrales, destaca su capacidad para establecer conexiones extensas con el cerebro huésped. Varios estudios, tanto en huéspedes neonatos como adultos, han demostrado la presencia de proyecciones procedentes de las neuronas ubicadas en el implante hacia áreas en el cerebro huésped (Bjorklund *et al.*, 1980; Jaeger y Lund, 1980; McLoon *et al.*, 1982; Lewis y Cotman, 1983; Gibbs *et al.*, 1986), así como proyecciones del huésped hacia el transplante (Sunde y Zimmer, 1983; Nothias *et al.*, 1988; Hohmann y Ebner, 1988; Gibbs y Cotman, 1987).

Aunque el establecimiento de conexiones entre el implante y el huésped puedan ser influenciadas tanto por el estadio de

desarrollo del cerebro huésped, como por la localización del implante así como por la técnica quirúrgica empleada, parece claro que tales conexiones pueden exhibir un alto grado de especificidad (Bjorklund y Stenevi, 1984).

De esta manera numerosas evidencias experimentales confirman el alto grado de integración que pueden llegar a alcanzar los implantes neuronales en diversas estructuras del SNC. En 1985 Anderson *et al.*, demuestran mediante técnicas inmunocitoquímicas, que neuronas septales implantadas en el hipocampo de roedor, marcadas positivamente con acetilcolinesterasa (AChE), forman sinapsis asimétricas con el tejido huésped y además reciben sinapsis de naturaleza no colinérgica. Wiegand y Gash (1985) demuestran mediante el empleo de peroxidasa de rábano (HRP) la existencia de proyecciones eferentes del núcleo supraquiasmático contenido en transplantes intraventriculares de hipotálamo anterior fetal. Isacson *et al.* (1985) describen la funcionalidad conductual y anatómica de transplantes neurales ubicados en el estriado (caudado-putamen) de ratas lesionadas previamente con ácido iboténico, su evaluación histológica revela altas densidades neuronales dentro de los transplantes y la expresión positiva de acetilcolinesterasa en toda la muestra.

En 1985 Ebner y Erzurumlu, demostraron que los transplantes de tejido embrionario, particularmente de células neocorticales en suspensión, son susceptibles de recibir inervación por parte de axones talamocorticales de huéspedes de diferentes edades, con este fin, fueron analizados individuos neonatos así como de tres y treinta días de edad. La reinervación más profusa se registró en los neonatos, en tanto que en los individuos de 30 días se detectaron proyecciones escasas. Observaciones en el sentido de que los transplantes de núcleo supraóptico y paraventricular hipotalámicos, colocados en el diencéfalo lateral de huéspedes adultos jóvenes, reciben inervación por parte de fibras noradrenérgicas provenientes del cerebro medio anterior (MFB), han

sido obtenidas por Silverman *et al.* (1985), quienes además evaluaron tales evidencias en varios tiempos post-transplante (6, 12 y 20 días), la reinervación más adecuada se registró en sujetos con 20 días post-implante, además detectaron mediante microscopía electrónica sinapsis axodendríticas y axosomáticas.

En 1985 Ross y Ebner, identificaron la capacidad diferencial de varios núcleos talámicos, complejo ventrobasal (VB) y núcleo posteromedial (POm) para inervar a transplantes de tejido neocortical embrionario ubicados en la corteza somatosensorial primaria (SI) de ratas adultas, dichos transplantes reciben aferentes desde POm pero no de VB, lo cual revela una sensibilidad diferencial de ambos núcleos hacia la axotomía y en consecuencia hacia la reinervación potencial. En un estudio realizado por Nothias *et al.* en 1988 donde fue analizado el desarrollo de aferentes monoaminérgicas procedentes del tejido adulto del huésped en transplantes fetales talámicos, se demuestra la presencia de fibras serotoninérgicas y noradrenérgicas en el interior de los transplantes, hacia los 8 días post-transplante. Este estudio demuestra la rápida incorporación de algunos sistemas de fibras de huéspedes adultos hacia los transplantes, y sugiere la integración funcional de éstos en los circuitos neuronales del tejido huésped, tras pocas semanas de desarrollo post-transplante.

Un estudio efectuado por Brasko y Das (1986) en el que se rastreó el desarrollo, la diferenciación y la integración de los transplantes, nos brinda algunos parámetros interesantes acerca de la paulatina formación de la interfase y posterior conectividad entre el implante y el huésped. Para tal efecto, fue transplantado tejido neocortical de fetos de 17 días de gestación en el hemisferio cerebelar derecho de ratas adultas, las cuales fueron sacrificadas y analizadas a diferentes tiempos. El análisis reveló la presencia de áreas densamente pobladas de tejido transplantado viable, en estrecha aposición con el huésped después de 24 horas post-transplante. Tras dos días de sobrevivencia el transplante

presentó un enlace parenquimal inicial con el cerebro huésped. Hacia los días 4 y 5 la formación de un verdadero neurópilo implante huésped constituía en sí una interfase real entre los dos tejidos. Por el día 15, los implantes alcanzaron gran crecimiento, presentaron agregados celulares interrelacionados a través de una fina red de axones que constituyó el sistema de conexiones intrínsecas del transplante. Finalmente entre los 15 y 90 días post-cirugía una serie de fibras provenientes de la región pontina penetraron en el implante alcanzando la capa medular del mismo.

LOS FACTORES TROFICOS Y LA INTEGRACION DE LOS TRANSPLANTES

El estado actual de las investigaciones en torno al papel que juegan los neurotransmisores y moléculas tróficas, en la promoción de la sobrevivencia neuronal, así como de la guía y crecimiento de axones y dendritas, no solo durante las primeras etapas de desarrollo, sino también en el individuo adulto como parte de los procesos de regeneración neuronal, permite pensar que tales factores intervienen de manera decisiva, en la sobrevivencia y adecuada integración de los transplantes de tejido nervioso fetal, los cuales a su vez son los responsables (como lo demuestran numerosos paradigmas conductuales) del restablecimiento de las funciones cognoscitivas y motoras de los sujetos transplantados.

A este respecto, se sabe que los tejidos cerebrales contienen factores tróficos que son activos *in vitro*, como promotores de sobrevivencia celular, crecimiento de neuritas y diferenciación tanto en neuronas de SNC como en SNP. Nieto-Sampedro y Cotman (1986) sugieren que factores neurotróficos específicos, incrementan su disponibilidad tras una lesión, contribuyendo en consecuencia, a la sobrevivencia y crecimiento de los transplantes. Así, aparentemente el cerebro responde al daño, a través de la producción de factores tróficos que incrementan la sobrevivencia celular y promueven el crecimiento de neuritas.

Aunados a los factores que sistemáticamente se hallan

implicados en la sobrevivencia, mantenimiento y diferenciación neuronal, tales como NGF (Factor de Crecimiento Neuronal), FGF (Factor de Crecimiento Fibroblástico), CNTE (Factor Neurotrófico Ciliar), BDNF (Factor Neurotrófico Cerebral), etc., en los últimos 5 años se ha venido desarrollando la tendencia cada vez mayor, hacia el estudio de los neurotransmisores no solo como moléculas implicadas en la transmisión de información, sino como importantes partícipes en la definición de la estructura de los circuitos en los cuales participan. Recientes hallazgos han demostrado el potencial de los neurotransmisores como reguladores de: motilidad del cono de crecimiento axónico durante el desarrollo (Goldberg y Kater, 1985; Haydon *et al.*, 1984); estructura sináptica en el cerebro adulto (Chang y Greenough, 1984; Desmond y Levy, 1983; Lynch, 1986) y neurodegeneración (Coyle *et al.*, 1983; Maragos *et al.*, 1987).

La mayoría de los datos acumulados hasta el momento, apuntan en dirección de que los efectos de los neurotransmisores sobre el crecimiento, son mediados a nivel del cono de crecimiento axónico. De esta manera, una terminal nerviosa constituye una estructura polimórfica, que durante el desarrollo funciona como un punto regulador para el crecimiento de neuritas (cono axónico), en tanto que en el adulto funciona como un punto de transmisión en la comunicación neuronal (terminal presináptica) Goldberg y Kater (1985).

La investigación en torno al papel que juegan los neurotransmisores en la regulación de la forma neuronal, se ha encaminado hacia el análisis de las alteraciones morfológicas que pueden ocasionar los neurotransmisores liberados por los axones aferentes en neuronas en desarrollo. El modelo de estudio que ha sido empleado para este fin, consiste en explantes de corteza entorhinal y neuronas piramidales hipocampales disociadas, en cultivo (Mattson, 1988).

En la extensa literatura que sobre transplantes se ha

generado en los últimos años, resulta relativamente frecuente el encontrar que los fenómenos de reconexión implante-huésped son adjudicados en buena medida a la presencia y actividad de factores tróficos. Uno de estos trabajos es el de Sharp y González (1985) en el que con el fin de probar la hipótesis de que los transplantes corticales fetales pueden disminuir la atrofia talámica causada por trastornos neonatales de la corteza frontal (CF), estos autores colocaron implantes unilaterales de corteza frontal de embriones de 18 días de gestación en un grupo de ratas a las cuales se les había disecado la CF al nacer, un segundo grupo de ratas recibió "gelfoam", el cual es un material sintético y biodegradable que en ocasiones funciona como sustento de fibras neuronales. Noventa días después, ambos grupos fueron sacrificados y las áreas talámicas respectivas fueron analizadas. Las áreas talámicas ipsilaterales al transplante de CF, mostraron dimensiones significativamente mayores que las correspondientes al grupo que recibió gelfoam; de acuerdo a los autores, este fenómeno puede deberse a los efectos tróficos del transplante sobre la corteza y el tálamo del huésped neonato. La administración de peroxidasa de rábano marcada con lectina (HRP-WGA) demostró la existencia de interconexiones transplante huésped. La presencia de proyecciones talámicas del huésped, sugiere que los transplantes constituyen un blanco para el desarrollo de neuronas talámicas.

ALGUNAS PERSPECTIVAS

Las investigaciones en torno a transplantes de tejido nervioso fetal han registrado avances significativos, particularmente en las últimas dos décadas, ampliando consecuentemente el panorama acerca de los diversos fenómenos que conforman la plasticidad del SNC de los seres vivos. Hoy en día, la combinación de la técnica de transplantes de tejido nervioso fetal con técnicas electrofisiológicas, histoquímicas, bioquímicas, de cultivo y más recientemente con la ingeniería genética, ha generado importantes aportaciones al estudio de la

recuperación funcional, la reconectividad, la neurobiología del desarrollo, así como de las interacciones tróficas entre el transplante y el huésped.

En el ámbito de la co-participación de los transplantes y la ingeniería genética por ejemplo, se han logrado progresos tales como los reportados por Olson y colaboradores (1989) quienes transplantaron exitosamente células secretoras de NGF (células recombinantes 3T3), que coadyuvaron a la restauración de sistemas colinérgicos previamente lesionados. Investigaciones de los mismos autores, han producido resultados positivos realizando transplantes intraoculares de células productoras de FGF obtenidas a través de recombinación. Asimismo se han transplantado fibroblastos genéticamente modificados, para producir NGF y promover así, la sobrevivencia y crecimiento de neuronas colinérgicas en la fimbria-fórnix previamente lesionada, permitiendo el restablecimiento de las proyecciones septohipocampales (Tuszynski *et al.*, 1989). Jinnah *et al.* reportaron en el mismo año, que transplantes de fibroblastos genéticamente modificados para producir L-DOPA, fueron implantados exitosamente en el núcleo caudado de ratas, a las que se les había lesionado previamente la vía nigro estriatal, restableciendo las deficiencias motoras. Finalmente Freed y colaboradores (1989) demostraron que la transferencia genética mediada por retrovirus, puede ser usada para producir tirosin-hidroxilasa (TH), enzima que participa en la biosíntesis de las catecolaminas, en líneas celulares eucarióticas, y que la proteína producida es altamente semejante a la enzima natural.

Estos experimentos sugieren que las líneas celulares producidas mediante recombinación genética tienen un gran potencial para producir efectos funcionales una vez que han sido transplantadas al interior del SNC (Freed *et al.*, 1989). De manera que el conocimiento acerca de la participación de numerosos factores tróficos y neurotransmisores en el desarrollo y funcionamiento del SNC, puede ser empleado exitosamente para generar nuevas estrategias terapeúticas encaminadas a prevenir o

retardar el avance de enfermedades neurodegenerativas, a través de la implantación de células genéticamente modificadas para proveer al sistema nervioso de los elementos necesarios.

Así, los recientes hallazgos de la recuperación morfológica y funcional de estructuras nerviosas dañadas, mediante la técnica de transplantes cerebrales, mencionados a lo largo de esta introducción, abre una nueva e interesante perspectiva a las neurociencias, así como al tratamiento neurológico. En el presente trabajo nos proponemos mostrar algunas evidencias experimentales sobre la recuperación mediante transplantes cerebrales, de procesos cognoscitivos que habían sido perdidos debido a lesiones previas en regiones del SNC, así como del restablecimiento morfológico de las estructuras dañadas.

Para demostrar lo anterior, hemos utilizado el modelo conductual conocido como Condicionamiento Aversivo a los Sabores, el cual ha tenido un gran impacto en la investigación neurobiológica, e inclusive en la aplicación clínica (Braveman y Bronstein, 1985).

EL CONDICIONAMIENTO AVERSIVO A LOS SABORES

En nuestro laboratorio hemos utilizado el modelo conductual de Condicionamiento Aversivo a los Sabores (CAS), originalmente propuesto por John García en la década de 1960; para evaluar la recuperación de los transplantes de tejido cerebral en ratas previamente lesionadas. García *et al.* en 1985 propusieron un modelo de condicionamiento en el que involucraron a los mecanismos de defensa externos e internos que se desarrollaron en los vertebrados como consecuencia de las presiones selectivas dentro de la cadena alimenticia (García *et al.*, 1955).

Los estímulos pertenecientes al sistema externo como son sonidos, choques eléctricos, mordeduras etc., y los asociados con el sistema interno, comò es la irritación gástrica, son integrados en sustratos anatómicos independientes en el SNC (García *et al.*,

1985; Bermúdez-Rattoni, 1986).

Dentro del sistema interoceptivo, el CAS desarrollado por García, ha sido desde la década de 1960 un método utilizado ampliamente en gran variedad de especies. En este tipo de condicionamiento, el gusto se presenta como estímulo condicionado (EC), el cual es seguido por irritación gástrica, como estímulo incondicionado. Una vez que el animal ha integrado esta información, generalmente desarrolla una aversión al gusto cuando se le presenta nuevamente este estímulo.

Entre las estructuras cerebrales superiores que se han asociado al CAS están: La amígdala (núcleos central y basolateral), (Nachman y Ashe 1974); la región septal (Mcgowan *et al.*, 1969); el hipocampo (Miller *et al.*, 1971); el hipotálamo (Gould y Proulx, 1972), y el área gustativa de la neocorteza (Braun *et al.*, 1972; Norgren y Wolf, 1975).

En mamíferos, las aferencias gustativas que ascienden a través de los nervios VII, IX y X, establecen relevos de primer orden en los tercios rostrales del núcleo del tracto solitario. Asimismo, este núcleo recibe aferencias viscerales provenientes de las ramas hepáticas del nervio vago que son sensibles a la irritación gástrica y del área postrema, la cual detecta la presencia de toxinas en la circulación periférica, así como del sistema vestibular, con sensibilidad a náusea producida por movimiento. El relevo gustativo de segundo orden, constituído por neuronas con capacidad de respuesta ante estímulos gustativos, se ha localizado en el área parabraquial del puente o área gustativa pontina, la cual emite proyecciones hacia el núcleo posteromedial ventral del tálamo (PMV). Las neuronas del núcleo PMV envían proyecciones gustativas hacia la corteza somatosensorial ventral e insular anterior en la rata (áreas 13 y 14 de la neocorteza de acuerdo a Krieg), denotada como Neocorteza Gustativa (NG) (Braun *et al.*, 1982).

Lasiter *et al.* (1982) han reportado la existencia de proyecciones directas entre el área pontina del gusto y la neocorteza gustativa de la rata, por su parte Saper (1982) y

Shipley (1982) han mostrado que las neuronas de la NG insular proyectan axones hacia el complejo parabraquial y la región caudal del núcleo del tracto solitario. Un año después, en 1983, Lasiter y Glanzman demuestran que las aferencias pontinas que recibe tanto el núcleo ventromedial tálamico como la NG tienen su origen en las mismas neuronas, es decir, colaterales axónicas del área gustativa del puente proyectan tanto hacia PMV como a NG.

El papel de cada uno de estos patrones neurales en la sensibilidad gustativa, ha sido inferido a través de métodos conductuales, electrofisiológicos y neuroanatómicos, lo que ha hecho posible el concluir con alguna seguridad, que mientras las zonas talámicas (PMV) y parabraquial pontina se hallan involucradas en la percepción del gusto, la neocorteza gustativa se relaciona fundamentalmente con la discriminación gustativa fina así como con la modulación conductual gustativa (Lasiter *et al.*, 1982).

Los trabajos efectuados por Wolf y Butcher (1982), Bermúdez-Rattoni *et al.* (1983) y López *et al.*, (en revisión), señalan que la acetilcolina juega un papel importante en el CAS. Existen evidencias en el sentido de que la neocorteza gustativa presenta considerable actividad colinérgica (acetilcolintransferasa y acetilcolinesterasa), así como de que el bloqueo farmacológico de la transmisión colinérgica provoca perturbaciones en la adquisición del CAS. Así, en estudios efectuados recientemente (López *et al.*, en revisión), se señala que los transplantes homotópicos neocorticales que promueven la recuperación del aprendizaje en el CAS, liberan ACh, en tanto que en el caso de los transplantes heterotópicos (corteza occipital), que no inducen recuperación funcional, no se registra liberación del citado neurotransmisor, lo cual sugiere la participación colinérgica en la recuperación conductual mediada por transplantes dentro del CAS.

Así pues considerando la serie de elementos mencionados en líneas anteriores en relación a la participación de los

transplantes de tejido nervioso fetal en los procesos de integración anatómica y restablecimiento funcional de los sujetos transplantados, así como a los estudios previos efectuados en nuestro laboratorio, a través de los cuales se ha demostrado la recuperación funcional y anatómica de la neocorteza gustativa por medio de transplantes de tejido nervioso fetal, mediante el empleo del modelo conductual del CAS, y habiendo analizado las conexiones que establece el transplante con diferentes estructuras cerebrales del huésped hacia la octava semana de desarrollo post-transplante; en el presente trabajo abordamos aspectos tales como el grado de inervación y el desarrollo citoarquitectónico temporal de los transplantes neocorticales, así como la posible participación de la reinervación y la maduréz citoarquitectónica de los mismos en los procesos de recuperación funcional de los sujetos transplantados.

MATERIALES Y METODOS

A fin de abordar los objetivos antes mencionados empleamos la siguiente estrategia metodológica.

SUJETOS

Para todos los experimentos se utilizaron ratas machos de la variedad Wistar, con pesos promedio entre 250 y 300 g, al inicio de cada experimento. Los animales fueron alojados en cajas individuales de acrílico y mantenidos en condiciones de luz obscuridad 12:12 y temperatura constante (22-24 °C).

LESION

Los sujetos fueron lesionados de forma electrolítica mediante técnicas estereotáxicas convencionales. los electrodos fueron construidos con barras aisladas de acero inoxidable, excepto en la porción apical (0.5 mm aproximadamente) y conectados a un estimulador que permitió el paso de una corriente de 2 mAmp durante 45 segundos.

Las coordenadas empleadas durante la cirugía de acuerdo al atlas estereotáxico de Paxinos y Watson (1982) fueron las siguientes: neocorteza gustativa (AP = + 1.2; L = $\pm$ 5; H = -5).

MEDICIONES CONDUCTUALES

Una semana después de practicadas las lesiones, los sujetos fueron entrenados en el procedimiento conductual de condicionamiento aversivo a los sabores que describiremos brevemente: los sujetos fueron privados de agua durante 24 hrs. y entrenados a beber en sus cajas diariamente en lapsos de 10 minutos por la mañana y 10 minutos por la tarde, durante 4 días. El volúmen de agua consumido se registró día con día empleando probetas graduadas de 50 ml provistas de tapones plásticos y tubos de cristal a manera de bebederos. Hacia el quinto día se efectuó la sesión de adquisición, en la que se substituyó el agua por una solución de LiCl 0.1 M, durante la sesión vespertina.

Posteriormente se efectuaron 5 ensayos más de consumo de agua (línea base de consumo), al cabo de los cuales se procedió a la sesión de prueba en la que se presentó una solución de NaCl 0.1 M. A esta sesión le siguieron 2 ensayos más de extinción, con 3 líneas base intermedias.

Se ha demostrado que las ratas no discriminan entre los sabores del NaCl y el LiCl, (Nachman, 1963).

TRANSPLANTE

Después de recibir el condicionamiento, los sujetos recibieron transplantes de tejido neocortical procedente de fetos de 17 días de gestación, para el caso de los transplantes homotópicos, y de tejido tectal o de corteza occipital, en los casos de transplantes heterotópicos. En cada caso, el bloque de tejido fetal correspondiente (1 mm^3 aproximadamente), fue introducido en una microjeringa Hamilton de 100 μl acoplada a un aparato estereotáxico, depositando el tejido en la ubicación correspondiente.

Tras varios tiempos de desarrollo post-transplante se procedió a efectuar el análisis conductual e histoquímico de los sujetos, el análisis conductual se efectuó de la manera descrita previamente.

ESTUDIOS HISTOLOGICOS

Una vez concluídos los estudios conductuales se procedió a procesar el tejido cerebral de los sujetos a diferentes tiempos post- transplante.

1) TRAZADORES DE CONEXIONES NEURONALES

Este tipo de métodos han sido ampliamente utilizados para demostrar el transporte retrógrado desde las terminales axónicas hasta el soma de la neurona.

1.a) Peroxidasa de rábano (HRP)

Los sujetos fueron anestesiados con hidrato de cloral al 10% (400 mg/Kg de peso), posteriormente el trazador fue administrado

estereotáxicamente en: neocorteza gustativa (AP = + 1.2; L = ± 5 y H = -5); complejo amigdalino (AP = - 2.5; L = ±5 y H = - 8) y tálamo (AP = - 2.5; L = ± 3.3; H = - 6) en volumenes de 0.5 µl de una solución de HRP (Sigma VI) y verde rápido al 2%, a una velocidad de 0.5µl/25', por medio de una microjeringa Hamilton de 1 µl. La aguja se mantuvo en su sitio durante 10 minutos después de la administración. 26 horas más tarde se procedió a la perfusión intracardiaca empleando: i) solución salina al 0.9% (250 ml/5'); ii) solución fijadora de glutaraldehído al 1.25% y paraformaldehído al 1%, en amortiguador de fosfatos 0.2 M, pH = 7.4 (500 ml/30'); iii) solución lavadora de amortiguador de fosfatos 0.2 M + sacarosa al 5%, a una temperatura de 4°C (500 ml/30'). Más tarde se procedió a la extración de los cerebros, los cuales fueron almacenados en una solución de amortiguador de fosfatos 0.1 M + sacarosa al 30%, por espacio de 24 hrs.. Finalmente los cerebros fueron cortados empleando un microtomo de congelación, las secciones (60 µm) fueron recogidas en buffer de fosfatos 0.1 M.

La detección de la peroxidasa se efectuó a través de su actividad enzimática oxidante sobre la tetrametilbenzidina (TMB), durante la incubación de las secciones de cerebro de acuerdo al siguiente procedimiento: Las secciones fueron transferidas a través de 6 cambios de agua destilada y desionizada a una temperatura de 1°C (1min/cambio), posteriormente fueron transferidas a la solución (A + B) donde permanecieron durante 25 min, al término de los cuales se añadieron 4 ml de peróxido de hidrógeno al 0.3% (25 min).

- Solución A: 92.5 ml de agua destilada
  5.0 ml de buffer de acetato
  0.5 g de gelatina
  100.0 mg de nitroprusiato de sodio
- Solución B: 1.0 ml de alcohol etílico absoluto
  5.0 mg de TMB

Finalmente se efectuaron 6 baños de 5 minutos en solución amortiguadora de acetato pH 3.3 y agua destilada al 5%, y se

procedió al montaje de las secciones en portaobjetos impregnados de gelatina. Después de 24 hrs. las laminillas fueron contrastadas con tionina (Mesulam, 1982).

1.b) Fluorogold

Recientemente hemos hecho uso de un trazador retrógrado con amplio poder de resolución llamado fluorogold, que cuenta entre sus ventajas la de poseer alta sensibilidad, no incorporación por fibras de paso, y compatibilidad con un gran número de técnicas histoquímicas (Alvarez-Buya, A., 1989, comunicación personal).

Durante la administración de este trazador los sujetos fueron anestesiados con hidrato de cloral al 10% (400 mg/Kg), la administración se efectuó estereotáxicamente con una jeringa Hamilton de 1 µl en los siguientes volúmenes: 0.3 µl en los núcleos ventromedial y ventroposteromedial talámicos, y 0.5 µl en el complejo amigdalino, de una solución al 2.5 % de fluorogold y solución salina fisiológica.

Cinco días después todos los sujetos fueron perfundidos por vía cardiaca con solución salina fisiológica (200 ml/rata) seguida de una solución de paraformaldehído al 4% y glutaraldehído al 0.1% (250 ml/rata). Los cerebros fueron almacenados en una solución de sacarosa al 20% durante 48 hrs., al cabo de las cuales fueron cortados (40 µm) y montados con solución de glicerol al 50%. Finalmente se procedió al análisis microscópico utilizando un microscopio de fluorescencia Nikon con un filtro de exitación UV -323 nm y emisión a -408 nm.

GOLGI RAPIDO

Este método ha sido empleado por más de un siglo para estudiar la arquitectura del SNC. En líneas generales, este método consiste en fijar pequeñas secciones del SNC en una solución de dicromato de potasio y ácido ósmico, y al cabo de un lapso de tiempo variable el tejido se transfiere a una solución diluída de nitrato de plata, finalmente las piezas son cortadas en secciones que son deshidratadas y colocadas en portaobjetos de cristal.

Así, durante los experimentos efectuados empleando esta técnica, los sujetos fueron anestesiados con pentobarbital y perfundidos por vía intracardiaca con formól al 10%, los cerebros fueron extraídos 24 hrs. después. Posteriormente se cortaron fragmentos de 4 mm^3 con especial énfasis en la zona correspondiente a la neocorteza gustativa. La solucion de fijación en la que fueron inmersos los fragmentos de cerebro, consistió en 4.5% de dicromato de potasio y 1% de tetróxido de osmio en agua destilada (3:1). Tras 10 días de fijación los cerebros fueron transferidos a una solución de nitrato de plata al 0.75% y almacenados en frascos obscuros durante 24 hrs., al cabo de las cuales el tejido fue "lavado" con etanol. Posteriormente el tejido fue deshidratado gradualmente permaneciendo por 24 hrs. en una solución de etanol absoluto y éter (1:1). Posteriormente el tejido fue embebido en soluciones graduales de nitrocelulosa del 2 al 30%, en un lapso de 5 días. En el paso correspondiente al último cambio de nitrocelulosa (30%), el tejido permaneció dentro de un desecador, en presencia de vapores de cloroformo durante 12 hrs.. Posteriormente los fragmentos fueron cortados en secciones de 120 μm las cuales a su vez fueron deshidratadas en alcoholes graduales. Finalmente las secciones fueron montadas con resina sintética.

HISTOQUIMICA PARA COLINESTERASA

Para este procedimiento los sujetos fueron anestesiados con pentobarbital y perfundidos por punción cardiaca con la misma fórmula descrita para el fluorogold. Posteriormente los cerebros fueron cortados en secciones de 40 μm que fueron montadas y embebidas en una solución de incubación que contenía yoduro de tiocolina. Al día siguiente las secciones fueron reveladas en presencia de sulfuro de azufre pH 5 y montadas en resina sintética.

TRABAJO I

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FETAL BRAIN TRANSPLANTS INDUCE RECOVERY OF MORPHOLOGICAL AND LEARNING DEFICITS OF CORTICAL LESIONED RATS

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INTRODUCTION

The recovery from brain injuring has recently been obtained using the fetal brain transplant technique in adult mammal brains. Thus, it has been established that transplanted neurons differentiate and make connections with the host brain (Frotscher and Zimmer, 1986). Moreover, there are studies that have been able to show biochemical and functional changes due to such transplants (Bjorklund and Stenevi, 1977; Drucker-Colín et al, 1984). Up until recently, some studies have shown cognitive function recuperation (Bjorklund and Stenevi, 1985; Dunnett et al, 1985).

In 1983 Labbe and coworkers, reported that rats with cortical transplants (E22) in the frontal neocortex were able to learn a spatial alternation task in fewer trials than lesioned control rats or rats with cerebellar implants. The recovery effects were seen just one week after transplantation. Moreover, it is noteworthy that cerebellar tissue transplants where atrophied while the frontal implants survived and were healthy and well integrated with the host tissue (Labbe et al., 1983; Stein et al., 1985). However, in 1987 Dunnett et al., tried to replicate the anterior report and found that neocortical grafts (E21) produced a short-lasting improvement in the t-maze alternation performance, therefore they concluded that "... the short-lasting recovery in delayed alternation performance is attributable to diffuse influences of the embryonic tissue on the lesioned host brain rather to a reconnection of the damaged circuites" (Dunnet et al., 1987).

Other studies have shown that the transplantation of either adult or embryonic frontal cortex accelerated the recovery of frontal cortex lesioned rats on a reinforced alternation task (Kesslak et al., 1986). The authors found that the rate of behavioral recovery correlates with the size of the surviving transplants. The recovery showed by animals with cortical grafts could be partially answered by the findings of Sharp and González (1986). In this study, they reported that there was an increase of survival thalamic neurons by

frontal cortical grafts as compared with those cortical lesioned animals. Moreover, they showed the existence of reciprocal connections between the thalamus and the graft by HRP-WGA injections.

The reasons for reconnection between host-graft are not well understood. Nevertheless, Chang and coworkers (1986) propose that while the factors determining the cortical arrange are intrinsic to the graft, the factors that determine the innervation between graft and host depends on the cellular environment which surrounds the grafted tissue. The first proposal has been demonstrated with grafts taken from frontal or occipital cerebral cortex that were placed into the occipito-parietal region of newborn rats. Results showed that the grafts developed normal pattern of lamination, with its original orientation, i.e.; the grafts had inverted orientation if they were placed upside down (Chang et al., 1986). The demonstration of the second comes from the same study in which the authors showed that regardless where the cortical area of the grafted tissue was taken, the transplants consistently received projections from those thalamic nuclei that normally innervated the adjacent host cortex. These results suggest that while immature cortical tissue may has an intrinsic, and perhaps autonomous, ability to develop lamination, the afferent and efferent cortical projections are most likely specified by extrinsic factors. However, for other authors the development of specific cell types and connectivity of the homotopic grafts, were mediated by intrinsic factors, as well as by the presence of some enzymes fundamental for the neurotransmitters synthesis (Smith et al., 1985). The authors indicated that the tissue taken at later stages of embryonic development (after cell migration and cortical plate formation) contains neurons that will express the synthetic enzyme for glutamic acid decarboxylase (GAD). When this happens, the GAD-labeled neurons in the surrounding host brain do not sprout into the transplants. On the other hand, neocortex taken at an early stage of development, in which the cell division and migration are just beginning, fails to express GAD and presumably contains no GABAergic neurons. Under these conditions the host GAD-positive neurons sprout profusely into the transplant. Therefore, the expression of some enzymes fundamental for the neurotransmitter synthesis, is very important for the innervation of host-graft (Smith et al., 1985).

In this regard, other studies had been made to investigate the innervation of grafts by host tissue. In 1986 Ebner and Erzurumlu, demonstrated that neocortical tissue transplants were innervated by thalamocortical axons of different ages hosts. With this purpose, the authors analized groups of newborn, and 30 days old rats, which received neocortical grafts. The most profuse reinnervation was observed in the group of newborn rats, while the subjects with 30 days showed fewer projections. One year before Ross and Ebner (1985) identified the differential capacity of several thalamic nuclei (ventrobasal complex; VB and posteromedial nucleus; POm) to innervate transplants localized in the somatosensorial cortex S_1. The grafts received afferents from POm but not from VB, which showed the differential capacity of both thalamic nuclei to innervate neocortical grafts. Finally, Hamasaki et al. (1987) transplanted lateral geniculate nucleus (LGN) from fetal rats into the visual cortex (VC) of neonatal rats, their results indicated that synaptic connections were established reciprocally between the transplanted LGN and the host VC. The presence of connections were observed through electro-physiological methods. All these researches, suggest the potential

plasticity of the neocortical grafts, as well as their capacity to reestablish reciprocal connections with the host tissue.

CONDITIONED TASTE AVERSIONS

A wide varieties of animals can associate flavor with toxic effects apparently as a result of the coevolution of protective mechanisms on the host species (See Garcia et al., 1985; Garcia et al., 1977). These flavor-illness associations have been demonstrated in many laboratories and with different species (Garcia et al., 1977). Thus, taste is readily associated with illness producing the conditioned taste aversions (CTA) after a single taste-illness experience. Unlike most other demonstrations of classical conditioning the delay between the taste (conditioned stimulus; CS) and the illness (unconditioned stimulus; US) could be an hour or more, and it is possible to have a strong taste aversion. The audio-visual signals are poor CS for a toxic US, they acquire little or no aversive properties following a single toxic US. In contrast, the audio-visual signal can be readily associated with the footshocks US, whereas tastes are poor CSs in shock avoidance conditioning (Garcia et al., 1982; 1985). This difference in conditioning has been termed cue-consequences specificity (Garcia and Koelling, 1966; Domjan, 1985).

One of the advantages to use this paradigm in the study of neural recovery by brain transplants, rests in the knowledge of the neural pathways involved in the CTA conditioning. Therefore, it is possible to know if fetal brain transplants could recover the previous damaged CTA pathways, and this could be correlated with functional recovery.

The neural mediation of conditioned taste aversion has been established with the use of anatomical, electrophysiological and behavioral methods (See Fig. 1). Thus, it has been established that the nucleus solitarius (NTS; the first gustatory relay) receives heavy visceral input from the hepatic branch of the vagus (sensitive to stomach irritating toxins) as well as inputs from the area postrema (sensitive to blood-irritating toxins) and the vestibular system (sensitive to nausea-causing motion). The NTS also receives primary taste afferents from the entire tongue via nerves VII and IX and from the larynx and parynx via X (Travers et al., 1987). Neurons responded to both gustatory and visceral stimuli are found in the pontine taste area of the parabrachial complex (second gustatory relay).

There are two main projections from the pontine taste area. One major projection of fibers passes to ventral forebrain structures, such as the amygdala, lateral hypothalamus, and the substantia innominata (Norgren, 1974). The second projection ascends ipsilaterally in the central tegmental bundle to the posterior ventromedial and ventromedial nucleus of the thalamus, a zone identified as a relay site for gustatory and lingual afferents. (Norgren and Leonad, 1973; Kiefer, 1985). The thalamic taste area projects to the gustatory neocortex (GN), a small band located in the anterolateral part of the cortex, 1 mm wide by 3 mm long along the rhinal sulcus in rat (Norgren and Wolf, 1975). Recently, Lasiter described a direct projection from GN to the amygdaloid complex via the internal capsule. The trajectory of these projections were established by application of horseradish peroxidase (HRP) in the GN which produced retrograde cellular labeling within the ipsilateral and basolateral amygdaloid nucleus (Lasiter, 1982).

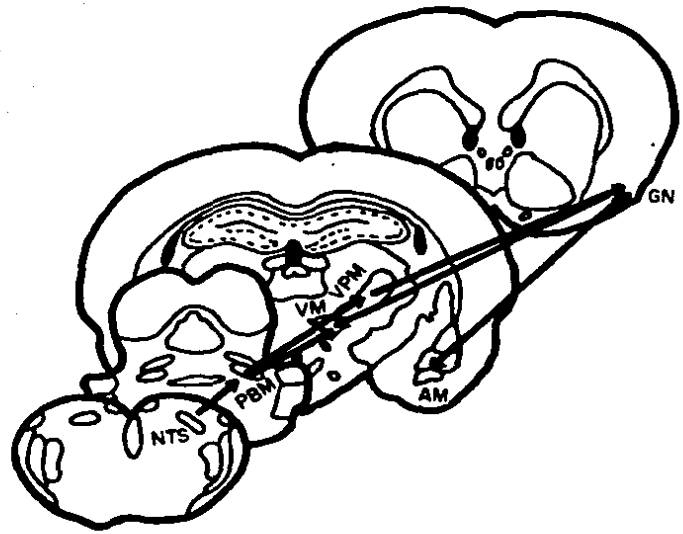

Fig. 1. Schematic drawing outlining the gustatory and visceral pathways in the rat. Abbreviations: (NTS) solitary nucleus, (PBM) medial parabrachial nucleus of the pons, (VM, VPM) ventromedial and posterior ventromedial thalamic nuclei, (AM) amygdala, (GN) gustatory neocortex.

Several studies have shown that the GN area is involved in the associative aspects of taste responding, but not in the hedonic responses to taste (Kiefer, 1985). Thus, rats lacking GN have a disrupted acquisition of taste aversions compared with the proper controls (Kiefer, 1985; Bermúdez-Rattoni et al., 1987 and Lasiter and Glanzman, 1985). Moreover, rats that were trained to avoid specific taste and followed by ablation of the GN, in the postoperative tests the lesioned animals showed no evidence of retention (Braun et al., 1981). On the other hand, the hedonic response of a lesioned GN rats appear to be normal. Since, it has been demonstrated that GN lesioned animals consume above water base-lines sucrose as well as low concentrations of sodium chloride. In addition, GN rats are able to reject quinine and acid solutions as normal rats do (Kiefer, 1985; Grill, 1985). These results indicated that GN integrity is not necessary for normal taste responsiveness. Moreover, it has been demonstrated that taste responsiveness remained intact even in decerebrate rats (Grill and Norgren, 1978).

BRAIN GRAFTS INDUCE RECOVERY OF TASTE AVERSION LEARNING

In a first series of experiments, we showed that the recovery of the lost ability to acquire taste aversions due to GN lesions is possible with homotopic cortical fetal brain transplants. Briefly, male Wistar rats, were randomly assigned to one of two groups. Large bilateral electrolytic lesions were made in one group to encompass the gustatory neocortex (Krieg's areas 13 & 14; See Fig. 1), the other group remained as unoperated control. Following post operative recovery the animals were deprived for 24 hrs., and trained to drink water daily during 5-minute trials for 10 days (See Fig. 3). The consumption volume was taken every day. On the acquisition trial, 0.1% saccharin was presented as a CS and followed 30 minutes later by intragastric LiCl (190 mg/kg) as US. An extinction trial was given after two water intake baseline measures; during extinction the CS was presented again, and the test volume scores were taken, this sequence was repeated once more.

Two days after the second extinction trial, the lesioned animals were divided randomly in four groups: One group received cortical homotopic grafts (GGN); other group heterotopic cortical occipital (GON) grafts; other group received heterotopic tectal (GT) graft; the last group remained without transplant as a lesioned control (LxGN). Seventeen-day old fetuses were removed from the abdominal cavity of pregnant rats. The fetal brains were taken (See Fig. 2), and the temporo-parietal area (above the rhinal sulcus) for the GGN group, occipital area for the GT group, and the tectal area for the GT group (See Seiger, 1985), were dissected under a microscope. The blocks of tissue were all then stereotaxically placed into the GN area with the same stereotaxic coordinates used to make the previous lesion (Bermúdez-Rattoni et al., 1987). After eight weeks of recovery, the four groups of rats were retrained in the same behavioral procedure described above. Results indicated that lesioned animals tested before transplantation showed the expected disrupted taste aversions when compared with the unoperated controls (Fig. 4). The postgraft results revealed that the animals with homotopic and heterotopic occipital grafts recovered the ability to acquire the taste illness association and were not significantly different from the control group. On the other hand, the groups which received the heterotopic tectal transplants or remained without transplant did not show any behavioral recovery (See Fig. 4; Bermúdez-Rattoni et al., 1987).

FETAL GN GRAFTS PRODUCED RECONNECTIVITY WITH THE HOST TISSUE

In other series of experiments we demonstrated with HRP histochemistry, that the transplants were able to reestablish connectivity with those areas that have been described as having normal connections with the GN. We followed the horseradish peroxidase protocol according to Mesulam (1982) technique and counterstained with thionine. The slices were examined and photographed under bright and dark field microscopy for the presence and location of retrogradely labeled neurons. Briefly, some control animals received a unilateral injection (0.5 ul) of HRP in the amygdala. The GN, occipital and tectal grafted animals received the same solution in the amygdala, always ipsilateral to the graft (Escobar et al., submitted).

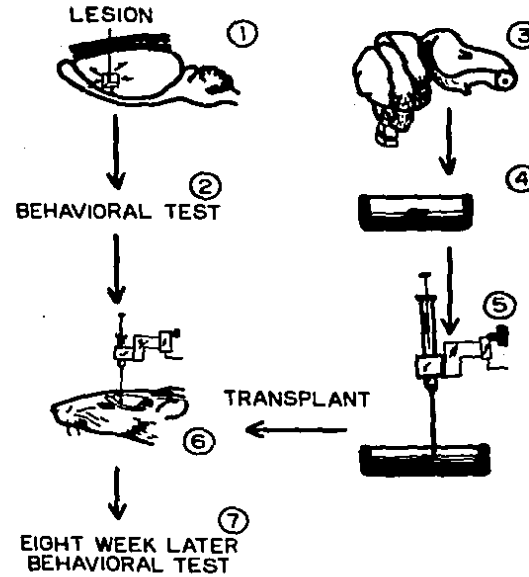

Fig. 2. Schematic representation of the methodological sequence. 1. Electrolytic lesions in the GN. 2. Behavioral test (see Fig. 3). 3, 4 and 5 transplant procedure; obtention of solid tissue from embryonic rats donnors; solid tissue block was put into a petry dish and aspirated by 100 ul Hamilton syringe. 6. Transplants were about 5 ul of embryonic tissue implanted with stereotaxic methods. 7. Eight weeks post-graft the behavioral training was given once more.

Results from HRP revealed that sections from control animals which received the enzyme in the amygdala, showed reaction product boundaries extended 1 mm in diameter surrounding the area of the tracer application (Escobar et al., submitted). HRP labelled cells were always found in the ipsilateral gustatory neocortex (Krieg's area 13 and 14), and in the ventromedial nucleus of the thalamus (See Fig. 5).

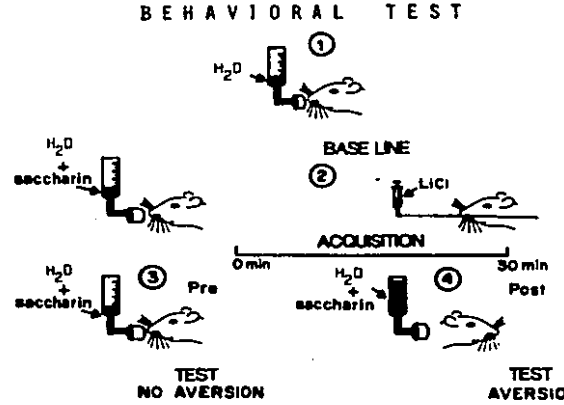

Fig. 3. Schematic representation of behavioral training sequence. 1. Training for 10 days to drink water for 5 minutes. 2. Acquisition day; presentation of saccharin disolved in the water (.1%) followed 30 min later with an infusion of LiCl (IG; 190 mg/kg). 3. Test day with saccharin in the water; Lesioned animals did not showed taste aversion, consuming similar quantities of the solution as baseline day. 4. Normal animals reject the solution, showing taste aversion.

The HRP histochemistry results support previous observations, that there is normal connectivity between amygdala and gustatory neocortex (Lasiter, 1982), since applications of HRP in the amygdala clearly produced labelled neurons in the ipsilateral gustatory neocortex of normal rats. Moreover, retrograde cellular labeling was found in the VPM of the thalamus, resulting from HRP applications in the amygdala. These results are in close agreement with those found by Lasiter (1982) and Kretek and Price (1974).

In general, the GN and occipital brain transplants appeared to be healthy and placed in the appropriate target area of the host brain. In both GGN and GON brain transplants we found scarce HRP labeled cells, although we found a good ammount of HRP labeled cells in the VPM and VM nucleus of thalamus in the same animals. The low density in the grafts have been previously described in studies that used HRP as a tracer for marking projections between host-graft tissues. Thus, a few labeled cells have been found when fetal brain transplants have been made in the hippocampus, occipital and somatosensory cortex, (Bjorklund and Stenevi, 1979; Jaeger and Lund, 1981; Kromer et al., 1980; Ross and Ebner, 1985). The reason of the low density of labeled cells has not yet been established (See, Jones, 1975). One hypothesis is that the fetal brain transplant is still under development and therefore their neurons are just starting to make connections with the host tissue. However, it has been shown that developing neurons are more efficient for incorporating peroxidase (Kristensson and Sjostrand, 1972). It is possible therefore, that the fetal brain transplant makes connections with its host, although not in a complete and normal fashion.

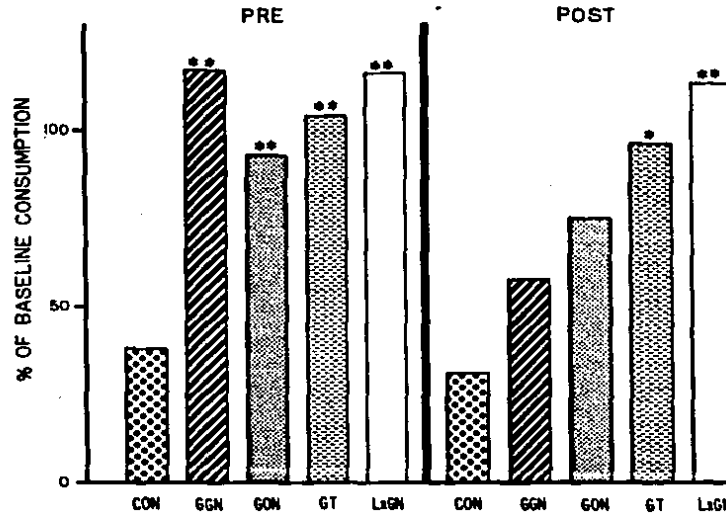

Fig. 4. The bars illustrate the amount of saccharin consumption by control, grafted and lesioned groups. Consumption is expressed as the percentage of each group's previous day water baseline, left side shows the results from one taste test trial prior to transplant. Right side shows the results of one taste test trial eight weeks after transplant; * $p < 0.05$, ** $p < 0.01$ (Dunnet test). (For description of groups see text).

On the other hand, the heterotopic tectal transplants did not integrate well with its host. There was a heavy glial invasion, necrosis, abundant vacuoles and very scant vascularization. Therefore, a complete lack of HRP labeled cells were found in the grafted tissue. Nevertheless, there were HRP labeled cells in the VM and VPM nucleus of the thalamus of the same rats (Escobar et al., submitted).

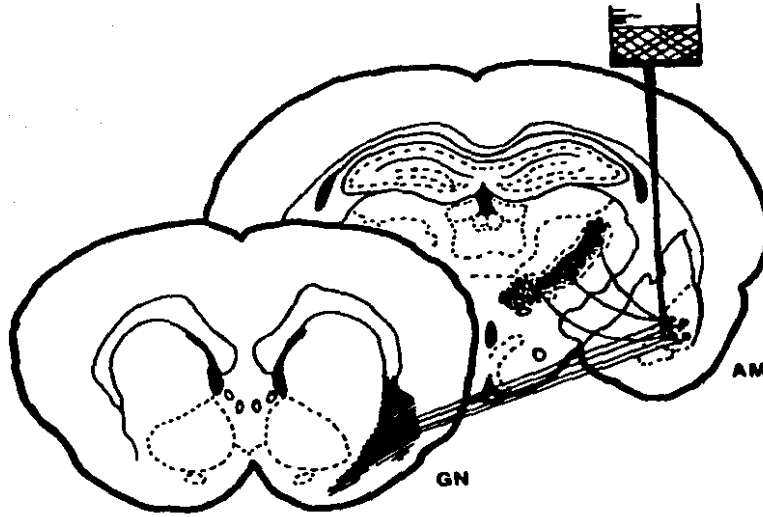

Fig. 5. Schematic representation of HRP injection in the amygdala (AM) and retrogradely labeled neurons within the gustatory neocortex (GN) and ventromedial nucleus of thalamus (VPM).

DISCUSSION

It is clear that gustatory neocortex lesions in rats produced disruption in the ability to associate the taste with its visceral consequences, these results have been reported several times (Braun et al., 1982; Kiefer, 1985). Moreover, the fetal brain transplants produced a significant recovery in the ability of lesioned rats to associate the taste with the visceral consequences (Bermúdez-Rattoni et al., 1987). The possibility of spontaneous recovery was excluded, because the animals with GN lesions that did not receive any

transplant, were unable to acquire the CTA after 8 weeks even with two acquisition trials (Yirmiya et al., 1987; Bermúdez-Rattoni et al., 1987). In contrast, in a previous report it was found that animals with lesions in amygdala showed spontaneous recovery after eight weeks post operation, when tested in taste aversion conditions (Bermúdez-Rattoni et al., 1987). Similar spontaneous recovery has been reported with large ablations of frontal cortex in an alternation task after six weeks postoperation (Dunnett et al., 1987).

Elsewhere we attempted to explain these functional differences between amygdala and GN. One possible explanation is that amygdala lesion produced reorganization of other elements in the neuronal network (Prado-Alcalá et al., 1978; Prado-Alcalá and Cobos Zapiaín, 1979). This idea has been demonstrated with functional alteration of the neostriatum. Thus, chemical alterations (i.e., microinjection of anticholinergic drugs) of the neostratum produces severe disruption of learned tasks (Prado-Alcalá et al., 1978). However, if the animals are overtrained, similar functional alterations do not produce disruption of the same learned tasks (Prado-Alcalá and Cobos Zapiaín, 1979). Therefore, it is possible to conclude that after the overtraining, the encoding necessary for the performance could be transferred to another neuroanatomical or neurochemical system. In our experimental conditions, overtraining could have been produced by repeated acquisition of taste aversion trials. Therefore, a plausible explanation for the differential effects between the cortical and amygdaloid lesions, is that for taste aversion learning the GN is a permanent memory store, whereas the amygdala only intervenes as an initial step storage for CTA (Bermúdez-Rattoni et al., 1987).

Our preliminary results showed that there is some recovery gradient regarding upon the place where graft tissue was taken. In Fig. 4 it is clear that the best behavioral recovery are from those animals which received homotopic cortical tissue (GGN), followed by those of occipital tissue (GON). Those animals which received tectal heterotopic tissue or remained without any transplant did not show any behavioral recovery. These results indicated that for taste aversion only cortical fetal brain tissue produced the recovery. Several authors employing other areas and different behavior tasks have reported that some tissue specificity is needed for anatomical and functional recovery. Stein and coworkers in 1985 made heterotopic cerebellar transplants into the frontal cortex and did not find any functional recuperation in a maze learning task. Moreover, they found a lack of integration of the cerebellar grafts with the host tissue as compared with the frontal graft integration. Similar results have been found when retina grafts are transplanted to a non-visual system location such as the cerebellum; the grafts do not form projections into the host brain, and the ganglion cells within the transplant degenerated (McLoon et al., 1985). In our results, the heterotopic tectal transplants did not integrate with its host, and there were heavy glial invasion, necrosis and very scant vascularization. Moreover, there were a lack of HRP labeled cells in the grafted tissue. The animals with tectum grafts did not recover the ability to associate taste with its visceral consequences (Bermúdez-Rattoni et al., 1987). These results give further support to the idea that some tissue specificity is needed for behavioral recovery.

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Fetal brain grafts induce recovery of learning deficits and connectivity in rats with gustatory neocortex lesion

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Three groups of rats showing disrupted taste aversion due to gustatory neocortex lesions, were studied. One group received a transplant of homotopic cortical tissue, another of heterotopic tectal tissue, obtained from 17-day-old fetuses. The third group remained without transplant as a lesioned control group. Comparisons of the taste aversion scores before and after graft, revealed that cortical grafted animals significantly improved the taste aversion, whereas those which received tectal grafts, and the cortical-lesioned controls did not. Moreover, results with horseradish peroxidase (HRP) histochemistry revealed that the homotopic, but not the heterotopic, brain transplants were able to re-establish connections with amygdala and with the ventromedial nucleus of the thalamus areas who normally kept connectivity with the gustatory neocortex. These results support the hypothesis that fetal brain transplants can re-establish cognitive functions, as well as connectivity with its host tissue.

The fetal brain transplant technique has been used to study recovery of previously damaged brain regions of the adult rat[5]. It is well known that fetal brain transplants establish functional and anatomical connections with the host brain[2,3,9]. Thus different behavioral procedures have been employed to study recovery of cognitive functions with fetal brain transplants[1,10,22].

One model that has been widely used in the study of learning processes is the conditioned taste aversion[12]. In this paradigm, normal rats acquire aversions to a taste cue conditioned stimulus (CS) when it is followed by illness as an unconditioned stimulus (US).

The anatomical pathways for CTA learning have been established with the use of anatomical, electrophysiological and behavioral methods[6,17]. For example, neurons responding to both gustatory and visceral stimuli are found in the pontine taste area of the parabrachial complex[17,31]. From this area, there are fibers of taste-response cells that ascend to the posterior ventromedial and ventromedial nucleus of the thalamus (VPM and VM) where they make synapsis with cells that in turn project fibers to the gustatory neocortex (GN)[17,24]. Recently, Lasiter[23] described a direct projection from GN to the amygdaloid complex via the external capsule. The trajectory of these projections were established by application of horseradish peroxidase (HRP) within the GN, which produced retrograde cellular labeling within the ipsilateral and basolateral amygdaloid nuclei[23]. Furthermore, the involvement of this area in CTA learning is demonstrated by the observation that lesions of GN, disrupt both acquisition and retention of a learned taste aversion[17,24]. Moreover, it has recently been shown that fetal brain transplants placed into the lesioned GN produced a recovery of previously lost CTA[1,32].

This report comprises two experiments. In a first experiment, we attempted to demonstrate that the

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recuperation of the lost ability to acquire taste aversions due to GN lesions, is possible with cortical but not with tectal fetal brain transplants. In a second experiment, we demonstrated, using HRP histochemistry, that the transplants are able to re-establish connectivity with those areas that have been described as having normal connections with the GN.

Male Wistar rats, weighing 250–275 g, were randomly assigned to one of two groups. Large bilateral electrolytic lesions were made under pentobarbital anesthesia (50 mg/kg) to encompass the gustatory neocortex (Krieg's areas 13 and 14; stereotaxic coordinates: AP = +1.2, L = ±5, H = −5, from skull level) in 15 animals, and 6 sham-operated animals were used as controls (CON). Following post-operative recovery (8 days), the animals were deprived for 24 h, and trained to drink water daily during 5-min trials for 10 days. The consumption volume was taken every day. On the acquisition trial, 0.1% saccharin was presented as a CS and followed 30 min later by intragastric LiCl (190 mg/kg) as US. An extinction trial was given after two water-intake base-line measures; this sequence was repeated once more. During extinction the CS was presented again and the test volume scores were taken.

Two days after the second extinction trial, the lesioned animals were divided randomly in 3 groups: one group received cortical homotopic[1] grafts (GGN; $n = 6$), the other group received heterotopic (tectal)[28] grafts (GT; $n = 4$); the other group remained without transplant as a lesioned control group (LxGN, $n = 5$). Seventeen-day-old fetuses were removed from the abdominal cavity of pregnant rats. The fetal brains were taken, and the temporo-parietal area (above the rhinal sulcus) for the GGN group, and the tectal[28] for the GT group, were dissected under a microscope. The blocks of tissue were about 2 mm^3, then stereotaxically placed through a Hamilton microsyringe (100 μl), into the GN area with the same stereotaxic coordinates used to make the previous lesion. After 8 weeks of recovery, the 3 groups of rats were retrained in the same behavioral procedure described above.

At the end of the experiment all the rats were sacrificed and perfused with 10% formalin, their brains were excised and coronal sections (10 μm thick) were made and stained with thionine to determine the transplants' characteristics.

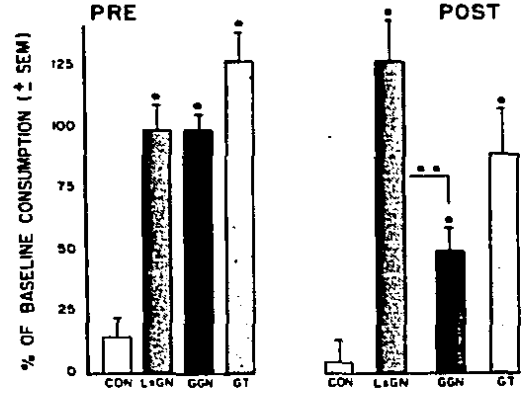

Fig. 1. The graph illustrates the amount of saccharin consumed by control, lesioned and grafted groups. Consumption is expressed as the perventage of each group's ptevious water baseline. Left side shows the results from one taste test trial prior to transplant. Right side shows the results of one taste test trial 8 weeks after transplant. $^{*}P < 0.05$ comparisons with CON group. $^{**}P < 0.05$ comparison between LxGN and GGN groups (Newman-Keuls' test).

In the second experiment, HRP histochemistry was made in 11 animals. Horseradish peroxidase (Sigma VI) was dissolved in Fast green solution 2% (0.4 mg/10 μl), that was always prepared fresh. Three control animals received a unilateral injection (0.5 μl) of this solution in the amygdala. Five GN-grafted and 3 GT-grafted animals received the same solution in the amygdala, ipsilateral to the graft.

The injections were made stereotaxically with a 1.0 μl Hamilton syringe, each injection lasted 25 min, and the needle was taken out 10 min after the end of the injection. After a 26-h survival period, rats were perfused intracardially with 300 ml of 20% sucrose in phosphate buffer (pH = 7.4), followed by 300 ml of 1.25% glutaraldehyde and paraformaldehyde in phosphate buffer (pH = 7.4); immediately the brains were removed and 24 h later they were sliced in coronal (60 μm) sections.

The slices were processed with tetramethylbenzidine (TMB) as a chromogen according to the Mesulam technique[26] and counterstained with thionine. Later, the slices were examined and photographed under bright- and dark-field microscopy for the presence and location of retrogradely labeled neurons.

Fig. 1 summarizes the results of pre- and postgraft CTA conditioning for all groups. Simple ANOVA

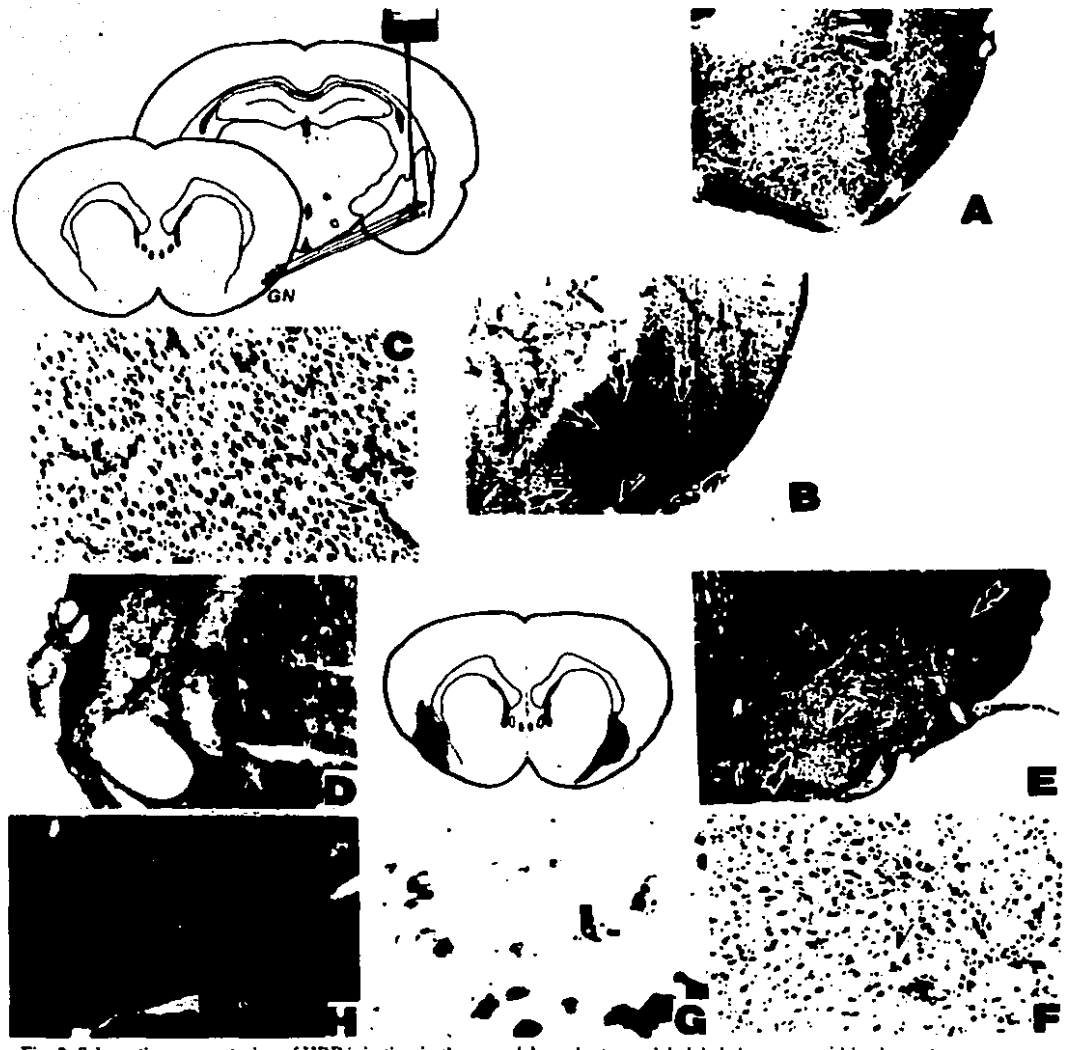

Fig. 2. Schematic representation of HRP injection in the amygdala and retrogradely labeled neurons within the gustatory neocortex (GN). A: a representative coronal section of amygdala HRP injection site. B: representative coronal sections of HRP-labeled control GN (arrows). D: the heterotopic GN grafts did not integrate well with its host. E: homotopic GN graft (indicated by arrows). C and F: representative cellular labeling within the gustatory neocortex of a control (C; arrowhead shows blood vessel from B) and grafted GN (F); note the lack of laminar pattern in F. G is a magnification of HRP-labeled cell marked with arrowhead in F. H is a dark-field illuminated magnification from another area of grafted GN. A, B, D, and E ×17.6; C and F ×44; H and G ×176.

was done on percentages from previous day baseline volume for each group, with post hoc group comparisons where appropriate using Student–Newman–Keuls' test[8]. We chose to analyze the data as percentages from baseline, because in the GN-lesioned animals the mean water baseline consumption was constantly lower (LxGN, 5.7 ± 0.1; GGN, 5.9 ± 0.5; GT, 6 ± 0.1) as compared with the unoperated controls. (CON, 7.95 ± 1.0) although there were no significant differences among lesioned groups. This effect has been reported previously by others[13]. During the pregraft first test trial the CON group showed a strong taste aversion and there were significant differences among groups ($F_{3,20} = 42.5$ $P < 0.001$). As expected, the GN-lesioned groups did not show taste aversions and all were significantly different from the control group ($P < 0.05$). During the second extinction trial (not shown in Fig. 1) there were no significant differences among groups, thus indicating that taste aversion was extinguished in the control group. The postgraft ANOVA comparisons revealed (Fig. 1, right) that there were significant differences among the groups ($F_{3,20} = 13.40$ $P < 0.001$). The GGN group recuperated the taste aversion although it still was significantly different from the CON group. The LxGN and GT groups did not recuperate the taste aversion after the transplants and their aversion scores were significantly different from the control group ($Ps < 0.05$). Moreover, the GGN group had significantly lower scores as compared with the LxGN group.

Histological examination revealed that the GN lesions involved the primary gustatory neocortical projection area[24], and the extension of the lesions were on average 1.2 mm in the dorsoventral plane and 2 mm in the anteroposterior plane[1]. The homotopic brain transplants appeared to be healthy and placed in the appropriate target area of the host brain (Fig. 2E). Results from HRP revealed that sections from

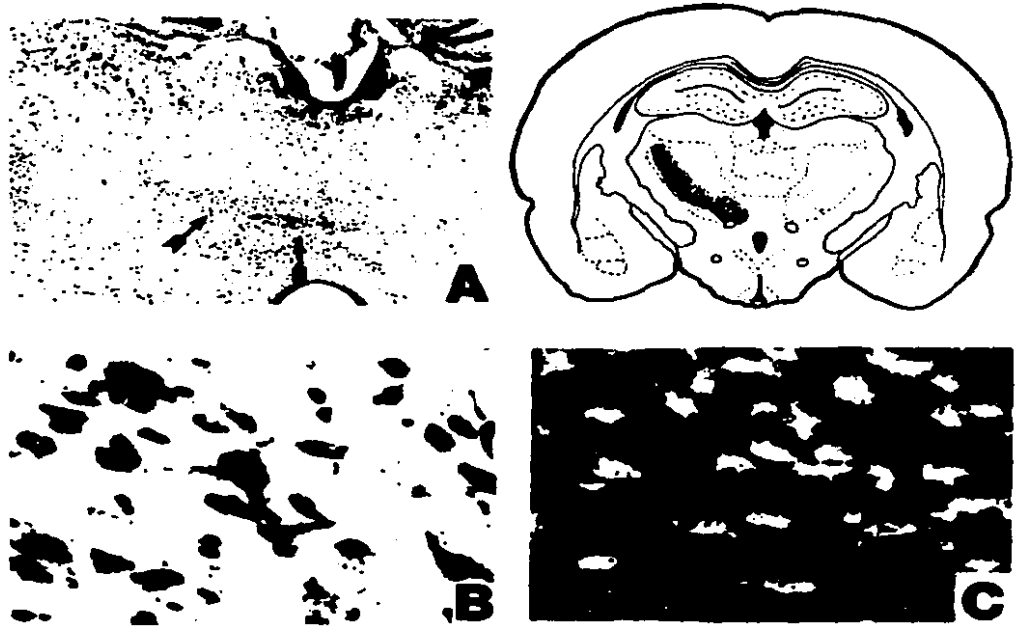

Fig. 3. A shows representative coronal section of HRP-labeled neurons (arrows) within ventromedial and ventroposteromedial (VM and VPM) nucleus of the thalamus. B is a magnification of A, showing HRP-labeled neurons. C is a dark-field illuminated magnification taken from the VM nucleus of a thalamus, from a grafted animal that received HRP injection in the ipsilateral amygdala. A ×4; B ×320; and C ×200.

control animals which received the enzyme in the amygdala, showed reaction product boundaries extended 1 mm in diameter surrounding the area of the tracer application (Fig. 2A). HRP-labelled cells were always found in the ipsilateral gustatory neocortex (Krieg's areas 13 and 14[19]; see Fig. 2B, C), and in the ventromedial nucleus of the thalamus (Fig. 3A, B).

In the animals that received homotopic cortical transplants and HRP in the ipsilateral amygdala, the detection of the enzyme was in neurons located in the interior of the grafts (See Fig. 2E–H), and in the VM and VPM nucleus of the thalamus (Fig. 3C). However, the cortical neuronal labeling density was much less than those normally found in the GN area of the controls (Fig. 2C, F).

From histological inspection, the heterotopic tectal transplants did not integrate well with its host (see Fig. 2D). There was a heavy glial invasion, necrosis, abundant vacuoles and very scanty vascularization. Therefore, a complete lack of HRP-labeled cells were found in the grafted tissue. Nevertheless, there were HRP-labeled cells in the VM and VPM nucleus of the thalamus of these same rats.

The HRP histochemistry results presented here support previous observations, that there is normal connectivity between amygdala and gustatory neocortex[23], since applications of HRP in the amygdala clearly produced labelled neurons in the ipsilateral gustatory neocortex of normal rats. Moreover, retrograde cellular labeling was found in the VPM of the thalamus, resulting from HRP applications in the amygdala. These results are in close agreement with those found by Lasiter[23] and Krettek[18], although in Lasiter's work a labeled amino acid was used to show the amygdala-cortical projection. In this study we demonstrated that the administration of HRP into the amygdala produced retrograde cellular labeling into the normal and grafted GN (see Fig. 2). These results indicate that the amygdala-cortical projections are bidirectional.

In the homotopic brain transplants we found few HRP-labelled cells (Fig. 2E, F), although we found a good amount of HRP-labelled cells in the VPM and VM nucleus of thalamus in the same animals. This low density has been previously described in studies that used HRP as a tracer for marking projections between host-graft tissues. Thus, a few labeled cells have been found when fetal brain transplants were made in the hippocampus, and occipital and somatosensory cortex[4,14,21,27]. The reason of the low density of labeled cells has not yet been established[15]. One hypothesis is that the fetal brain transplant is still under development and therefore their neurons are just starting to make connections with the host tissue. However, it has been shown that developing neurons are more efficient for incorporating peroxidase[20]. It is possible therefore, that the fetal brain transplant makes connections with its host, although not in a complete and normal fashion.

This hypothesis is supported by recent findings with Golgi-stained gustatory neocortical fetal brain transplants. Inspection of the Golgi-stained graft tissue revealed that there is indeed a heavy regeneration processes; immature neurons with a low number of dendritic spines, gliosis, a lack of cortical laminar pattern and a large number of vascular vessels in the graft[7].

The behavioral results of these experiments clearly show that the cortical fetal brain transplants produced a significant although not complete recovery in the ability of the lesioned rats to associate taste with visceral consequences, confirming observations made by ourselves as well as other authors[1,32]. The possibility of spontaneous recovery was excluded because the GN-lesioned group without transplant did not acquire CTA after the post-graft second acquisition trial. The heterotopic tectal transplants did not produce behavioral recovery, since it was similar to the LxGN group, indicating that some tissue specificity is needed for anatomical and functional recovery. Similar results have been found in other areas and with different behavioral tasks[25,29]. Thus, Stein and coworkers[29] made heterotopic cerebellar transplants into the frontal cortex and did not find any functional recovery in a maze-learning task. Moreover, they found a lack of integration of the cerebellar grafts with the host tissue as compared with the frontal graft integration. However, recently it has been suggested that the structural and morphological integrity of fetal brain transplants may not be essential for behavioral recovery after brain injury[11,16,30]. These authors have speculated that brain injury and/or brain transplants induced a release of neurotrophic substances, that can re-activate neural function and/or prevent injury-induced degeneration in the damaged host brain[11,16,30]. In our results, the possibility that neuro-

trophic factors may be involved in the functional recovery is low, although possible, since animals with tectal transplants showed very slight improvements in postgraft-acquired taste aversion learning as compared to the LxGN group (see Fig. 1). Therefore, if neurotrophic factors are involved, they need to be associated with homotopic cortical tissue. In summary, our results suggest that some morphological recovery may be sufficient for the acquisition of taste aversion learning, though the possibility that neurotrophic factors are also involved has not been ruled out. We are currently investigating this issue.

In any case the present study demonstrates that homotopic cortical (but not tectal) fetal brain transplants can restore the association of taste with its visceral consequences, while in addition, it demonstrates with HRP histochemistry that cortical, but not tectal, fetal transplants are able to re-establish connectivity with its host brain tissue.

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TRABAJO III

Enviado a Behavioral Neurobiology

TIME-DEPENDENT RECOVERY OF TASTE AVERSION LEARNING BY FETAL BRAIN TRANSPLANTS IN GUSTATORY NEOCORTEX LESIONED RATS

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ABSTRACT

We recently showed that fetal brain transplants produced a significant recovery in the ability of gustatory neocortex lesioned rats to learn a conditioned taste aversion task. In this report we studied the capability of gustatory neocortex fetal brain transplants to produce behavioral recovery at different times. Four groups of male Wistar rats showing disrupted taste aversions due to gustatory neocortex lesions were employed. The lesioned animals received fetal cortical grafts, obtained from 16-day old fetuses, and were retrained in the behavioral procedure after 15, 30, 45 and 60 days post-graft. It was found a very good functional recuperation at 60 days, slight recovery at 45 and 30 days and a poor recovery at 15 days post-graft. Results with HRP histochemistry revealed that at 30, 45 and 60 days post-grafting there were increased connections with the ventromedial nucleus of the thalamus and with the amygdala. At 15 days post-grafting there was absence of HRP labeled cells. In addition, behavioral recovery was correlated with increased acetylcholinesterase activity detected histochemically and with morphological neuronal maturation showed by Golgi staining. These results suggest that morphological maturity and reconnectivity between grafts and host tissue are needed for behavioral recovery in gustatory neocortex lesioned rats.

INDEX WORDS: Conditioned Taste Aversion; Grafting; Gustatory Neocortex; Horseradish Peroxidase; Golgi Stain; AchE.

Running Title: Time-dependent recovery by fetal brain grafts.

The fetal brain transplant technique has been used recently as a very effective tool to ameliorate functional and behavioral deficits produced by either mechanical (Kesslak, Nieto-Sampedro, Globu & Cotman, 1986; Woodruff, Braisden, Whittington & Benson, 1987), chemical (Issacson, Dunnett & Bjorklund, 1986; Kesslak, Walencewicz, Calin, Nieto-Sampedro & Cotman, 1988) or degenerative injuries to adult mammal brain (Collier, Gash & Sladek, 1988; Fine, Dunnett, Bjorklund & Iversen, 1985; Huang, Kissane & Hawrylewics, 1987).

Conditioned taste aversions (CTA) has been used widely as a model for the study of learning processes (García, Lasiter, Bermúdez-Rattoni & Deems, 1985). In this model animals can acquire aversion to a taste cue (conditioned stimulus; CS) when it is followed by gastrointestinal illness (unconditioned stimulus; US). The anatomical pathways involved in CTA have been extensively studied (for review see García et al., 1985; Kiefer, 1985). Briefly, the posterior ventromedial (VPM) and ventromedial nuclei (VM) of the thalamus receive afferents from the pontine taste area. These thalamic nuclei send fibers to both the gustatory neocortex and the amygdala (Kiefer, 1985; Lasiter & Glanzman, 1985); It has recently been described reciprocal connections between the amygdala and gustatory neocortex (Escobar, Fernández, Guevara-Aguilar & Bermúdez-Rattoni, 1989; Lasiter & Glanzman, 1985).

Lesions of the gustatory neocortex region (GN) in adult rats lead to a behavioral impairment in both acquisition and retention of conditioned taste aversions (Kiefer, 1985; Lasiter & Glanzman,

1985). It has been demonstrated that cortical fetal brain transplants induce recovery of taste aversion learning in rats with gustatory neocortex lesions (Bermúdez-Rattoni, Fernández, Sánchez, Aguilar-Roblero & Drucker-Colín, 1987; Escobar et al, 1989; Yirmiya, Zhou, Holder, Deems & Garcia, 1988).

The mechanisms by which the brain transplants produce functional recovery, are not well understood. In this regard, several authors explain the behavioral improvements after fetal brain transplants in previously lesioned animals, as being due to release of "trophic" factors (Labbe, Firl, Mufson & Stein, 1983). Other groups have pointed out that new connections between the graft and the host are responsible for the behavioral recuperation (Dunnett, Low, Iversen, Stenevi & Bjorklund, 1982). Kesslak and coworkers (Kesslak et al., 1988) reported that hippocampal but not glial transplants to adult rats produced partial recovery of forced-choice alternation task. These results suggest that morphological recovery is necessary for the functional recovery. In agreement with this hypothesis, we recently demonstrated using the horseradish peroxidase histochemistry technique (HRP), that cortical but not tectal brain transplants, were able to produce behavioral recovery and re-established connections with the amygdala and with the ventromedial nucleus of the thalamus (Escobar et al., 1989).

Several authors have found connections between the cortical grafts and the host brain. Floeter and Jones (1985) reported that the cortical transplant projected fibers to the thalamus, the contralateral cortex, the striatum and the hippocampus. Castro

and coworkers observed ten months after cortical grafting, that the transplanted tisue had received fibers from the basal forebrain, locus coeruleus and raphe (Castro, Tonder, Sunde & Zimmer, 1988). These results clearly show that the cortical grafts are able to survive in the brain parenchyma, and to establish aferent and efferent connections with the host tissue (Ebner, 1988; Escobar et al., 1989).

In the present study we report the time course of the behavioral recovery induced by gustatory neocortex transplants after GN lesions, as well as the time course of the appearance of connections with the amygdala and thalamus. In addition, we report the time course of appearance of acetylcholinesterase reactivity and the development of grafted tissue using the Golgi staining technique.

METHODS

Subjects

Fifty four male Wistar rats weighing 250-280 g were individually housed in plexiglas house boxes and had ad-lib access to food and water, except during the CTA procedures (see below), and kept on a strict 12:12 h light-dark cycle (08:00 h on; 20:00 h off).

Surgery Procedure.

Large bilateral electrolytic lesions were made under pentobarbital anesthesia (50 mg/kg) to encompass the gustatory neocortex (AP=± 1.2 mm, L±5.3 mm, V-5 mm) in thirty experimental animals. Lesions were made by passing a direct anodal current

(1mA/60 sec) through a stainless steel electrode coated with epoxyle except for the cross section of the tip. Twenty four animals were used as unoperated controls.

Behavioral Procedure.

Following postoperative recovery (7 days) the experimental and control animals were water deprived for 24 h and trained to drink water in their home cages, daily during a ten minutes period in the morning and an equal period in the afternoon. The water consumption volume was measured everyday with 50 ml calibrated test tubes equipped with a rubber stopper and glass drinking spout. Water consumption was recorded to the nearest 0.5 ml. On days 1-4, animals were allowed access to water for 10 min each, morning and afternoon. On day 5, (the acquisition trial) 0.1 M of LiCl was presented instead of water in the afternoon period. An extinction trial was given after five water intake baselines measures. On day 8 in the afternoon (the first extinction trial) 0.1 M of NaCl was given instead of the LiCl. Two extra extinction trials (days 10, 12) were given, with three water-intake base-lines measures, in between (see Bermúdez-Rattoni et al., 1987). It has been demonstrated that rats cannot discriminate between the NaCl and the LiCl flavor (Nachman, 1963).

Transplant Procedure.

After the behavioral tests, the experimental animals were divided randomly in to 4 groups (see below), and received homotopic cortical fetal brain transplants as previously

described. Briefly, 16-day old fetuses were removed from the abdominal cavity of pregnant rats under barbiturate anesthesia (50 mg/Kg). The fetal brains were removed, and the temporo-parietal area (above the rhinal sulcus) was dissected under a microscope. The tissue (about 2 mm^3) was aspirated into a 100 ul Hamilton microsyringe and then stereotaxically injected into the GN area with the same stereotaxic coordinates used to make the previous lesion. The experimental animals were randomly assigned to be retrained for CTA at fifteen (G15; n=8), thirty (G30; n=8), forty five (G45; n=6) or sixty (G60; n=8) days postgraft, using the procedure described above. Control groups (C15; n=6, C30; n=6, C45; n=6, C60; n=6), were handled in parallel.

Histological Procedure

At the end of the experiment, horseradish peroxidase (HRP) histochemistry, acetylcholinesterase (AChE) histochemistry and Golgi impregnation were made each in at least two rats per group

HRP Histochemistry. Horseradish peroxidase (Sigma VI) was dissolved in fast-green solution 2% (0.4 mg/10 ul). Sixteen GN grafted subjects, (4 from each experimental group) received an unilateral injection (0.5 ul) of the HRP solution in the amygdala (n=2) or in the thalamus (n=2) ipsilateral to the graft. In addition, two control animals from group C60 received an unilateral injection of the same solution in the amygdala and three rats from the same group in the thalamus. The injections were made stereotaxically with a 1.0 ul Hamilton syringe. Each

injection lasted 25 min and the needle was taken out 15 min after the end of the injection. After a 26 h survival period, rats were perfused transcardially with 300 ml of 1.25% glutaraldehyde and paraformaldehyde in phosphate buffer (pH = 7.4), followed by 300 ml of 20% sucrose in phosphate buffer (pH = 7.4); twenty four hours later, the brains were removed and sliced in coronal (60 um) sections.

The slices were processed with tetramethylbenzidine (TMB) as a cromogen, according to the Mesulam technique (Mesulam, 1982) and counterstained with thionine. The slices were subsequently examined and photographed under bright and dark field microscopy for the presence and location of retrogradely labeled neurons.

Golgi Stain. Six animals from groups G60, G30 and G15 (2 each) were anesthetized with pentobarbital, perfused through the heart with 10% neutral buffered formalin and the brains removed the following day. In each rat a 4 mm wide coronal block of tissue including the gustatory cortex was prepared for the rapid-Golgi technique. The immersion-fixation solution consisted in 4.5% potassium dichromate ($K_2Cr_2O_7$) and 1% of osmium tetroxide in distilled water (3:1). After 10 days of fixation the solution was poured off and the tissue drained briefly on absorbent paper and transferred to a 0.75% silver nitrate ($AgNO_3$) solution stored in brown glass bottles. Twenty four hours later the tissue was removed from the silver solution, drained briefly on absorbent paper and then washed with an ethanol-impregnated brush. Then the tissue was gradually dehydrated with ethanol and stored for 24 hours in absolute ethanol and ether. Following that, the

tissue was gradually exposed to gradually more concentrated solutions of nitrocellulose (from 2% to 30%) over the course of not more than 5 days at the maximum. The blocks were embedded in low viscosity 30% nitrocellulose and hardened overnight in a container with chloroform vapors.

Serial sections were cut at 120 microns thickness on the sliding microtome, dehydrated in ethanol (70%, 80% and 95%; 10 min each), and passed through 98% isopropanol and 98% terpineol (10 min each). The sections were transferred to reagent grade xylene and mounted with synthetic resin.

Acetylcholinesterase histochemistry.

The remainder of the animals (2 from each experimental group) were anesthetized with pentobarbital and perfused transcardially with the same formula described above for HRP injections. The brains were cut coronally (40 um thickness), mounted and then immersed in the incubating solution described by Paxinos & Watson (25). The following day the slices were developed in sodium sulfide (pH 5) and mounted with synthetic resin.

RESULTS

Behavior.

Oneway ANOVA was done on the test day consumption volume for all groups, with post hoc group comparisons were appropriate using the Student-Newmann-Keuls'test (Fig.1). During the pregraft test trial, there were significant differences among groups >F(7,51)=11.4, P .001<. As expected, the four control groups

showed strong taste aversions in the first test trial. The experimental (with GN lesions) groups showed significant disrupted taste aversions when compared with their own controls (p's 0.05). Postgraft ANOVA comparisons (Fig 1., right) revealed that there were significant differences among the groups >F(7,51)=6.88, P 0.001<. The control groups again showed a very good taste aversions. The G15 group showed a disrupted taste aversion, consuming significantly more NaCl solution when compared with its own control (P 0.05). The G30, G45 groups, consumed more saline solution than their respective control, although there were not significant differences. The G60 group showed a strong water intake suppression in the presence of the CS, which was similar to its own control.

In addition, paired t tests between pre and postgrafts volume consumption were done. The postgraft scores revealed that groups G30, G45 and G60 showed a significant aversions as they reduced their water consumption in the presence of the CS when compared with their pregraft scores (p's 0.05). In contrast, group G15 showed disrupted taste aversions pre and postgraft, as they had similar NaCl water consumptions.

INSERT FIGURE 1 ABOUT HERE

HRP Histochemistry.

The analysis of the brain tissue injected in the thalamus or amygdala with HRP in the G15 experimental animals showed that there were not labeled HRP-cells in the grafted tissue. In the

graft tissue of G30 animals there were scarce labeled HRP neurons. In contrast, in the 45 and the 60-days graft tissue, there was found a great number of labeled neurons, though not as many as in control tissue, as we have described previously (Table 1; Fig. 2) (Escobar et al., 1989). In all the grafts in which there were found HRP labeled cells, the cell distribution inside the transplant did not follow any distinguishable pattern.

INSERT FIGURE 2 AND TABLE 1 ABOUT HERE

Golgi Stain.

The Golgi stain results were obtained from 6 adult brains with fetal brain transplants. We observed differences at each age of the transplanted tissue. The difference in the tissues taken at different times had different stages of neuronal development and maturation. In general, the grafted tissue in all experimental groups showed a neural reorganization in both tissues (grafted and host) with a greater neuronal density in the transplanted tissue particularly in those of 60 days. In general, the fetal transplants were adhered to the host tissue with abundant vascularization, great proliferation of glial cells in the transplant border as well as fibers that cross the interface. Chronological changes: Fifteen days: transplanted tissue showed scarce development of neurons and blood vessels. Round-shaped neurons appeared with few dendritic processes. Some of them had no spines at all in their dendrites (Fig. 3a). There were few glial cells in the border of the transplant. In an overall view

the grafts seemed to be an initial state of neuronal development, with an incipient vascularization process between transplant and host tissue. Thirty days: Graft tissue appeared to be in a more advanced stage of development (Fig. 3b). That is, neurons showed a great number of dendritic processes, growing in all directions from the cell body. The axons were apparent in the majority of the neurons. Blood vessels were found in the border and inside the transplanted tissue. Many pyramidal and multipolar neurons were found inside the transplant. Glial cells were found in many parts of the transplanted tissue, without any regular pattern. Sixty days: The transplanted tissue showed a great advance in the development of neurons and glial cells (Fig. 3c). Neurons presented multipolar, piriform and triangular shaped-somas; some of them had many dendritic spines. Neurons were surrounded by abundant vascularization in all parts of the transplant. We observed well developed glial cells, in close relation with the neurons. In general, neuronal vascular and glial components were observed in different parts of the transplanted tissue. The fibers were more abundant in the border of the transplant, however a lack of cortical lamination as compared with adjacent host tissue was noted.

INSERT FIGURE 3 ABOUT HERE

Acetylcholinesterase Reactivity.

We found that the 15-day animals showed some labeled cells and there were few processes in the transplant. In the 30, 45 and 60

day groups there was an increased number of AchE fibers inside the transplants (Fig. 4). These fibers formed patches along the grafts. The 15-day post-transplants groups did not show these AchE patches, since there were few AchE stained fibers (Fig. 4a). We could not observe any difference in the number of cells among the different transplants groups, although there were an increased AchE reactivity within the G30, G45 and G60 (Fig. 4).

INSERT FIGURE 4 ABOUT HERE

DISCUSSION

The behavioral data obtained in these experiments showed that it took the grafts at least 30 days to start producing functional recovery in the host animals. During the initial fifteen days post transplant, the subjects did not show any recuperation in the CTA paradigm (Fig. 1). After thirty and forty five days post transplant the animals were able to learn the aversive response to the noxious stimulus. At sixty days postgraft the behavioral recovery was almost complete (G60; see Fig. 1), as the grafted group show any significant differences with its own control (Fig. 1). The time-dependent behavioral recovery was accompanied by time-dependent histological changes. At 15-days post-graft the cortical transplants did not establish any demonstrable connections with thalamus nor with the amygdala (table 1). In the 30, 45 and 60-days post-graft groups, the brains showed increased connections to both the VPM and the amygdala (see table 1). The

neurons of the transplant from these groups also showed a more mature cell morphology. In the groups with 30 and 60 days postgraft, the Golgi stain revealed that cell bodies were more mature, since they had more dendritic processes with more spines. These results are in agreement with those that have used a mature (more than 60 days) cortical fetal brain transplants (Yirmiya et al., 1988). In contrast, the 15 days postransplant group showed an immature cell morphology with a few number of dendritic spines observed with the Golgi staining technique (see Fig. 3).

Our results suggest that some maturation of the transplanted tissue accompanies behavioral recovery. The maturity of the neurons can be determined by the number of its connections, which included in part those established between the transplant and the host. Recently, some authors have reported the establishment of connections between cortical grafts and the thalamus in neonatal rats after two to four months after transplantation (Castro, Zimmer, Sunde & Bold, 1985). Moreover, other studies have also showed that the thalamus of the adult brain could only establish few connections with the cortical transplants, from eight to twenty eight weeks after transplantation (González, Sharp & Loken, 1988). We had previously demonstrated that 60 days transplants of the gustatory neocortex could establish connections with the VPM and the amygdala, although the number of labeled cells were not as numerous as in control animals (Table 1; Escobar et al., 1989). In the present paper, the results suggest that the transplanted neurons required more than 15 days to start making connections with both the thalamus and the

amygdala. In addition, with the use of the Golgi impregnation, in the 60 days grafts we were able to see some fibers crossing the boundaries of the transplant into the host tissue, indicating a dynamic process of interaction between the transplant and the host tissue. However, there was not a laminar arrangement in the grafts as in the normal host tissue. These results are in agreement with others that found partial functional recovery without a normal laminar arrangement of fetal cortical brain transplants (Mufson, Labbe & Stein, 1987).

It is clear from these results that morphological recuperation is necessary to obtain a functional recovery in gustatory neocortex lesioned rats. This observation is supported by our recently published paper, in which, we were able to show that homotopic cortical but not heterotopic tectal fetal brain transplants could restore the associations between taste cues and illness. Moreover, it was demonstrated with HRP histochemistry that the homotopic, but not the heterotopic, fetal brain transplants were able to re-establish connections with the host tissue (Escobar et al., 1989).

The demonstration of the AChE expression in the transplant in our results is supported by previous observations (Hohmann & Ebner, 1988). Thus, other authors (Park, Clinton & Ebner, 1984) found AchE expression after 7 days of cortical transplantation. However, it was only after 2 months that they found AchE reactivity that was similar to the cortical host tissue of cortical transplantation (Park et al., 1984). In this paper, we are showing the time course of AchE graft expression. A great reactivity of the 15-day soma in the graft, but with few

processes were observed. The number of all processes were increasing in the 30, 45 and 60-day post-graft, though the somas showed decreased AchE reaction with the time. Some authors have proposed that the neurotransmitters enzymes have some axon guidance effects. That is, Robertson (1987) has demonstrated transient expression of acetylcholinesterase in the developing thalamo-cortical system, but such transient expression lasts for only three weeks and then decline to normal adult levels when the thalamo-cortical connections have been well established. Another, but not excluding, explanation is that there are in growth of axons from the basal forebrain or from the nucleus basalis magnocellularis to the cortical grafts as previously demonstrated by Ebner et al. (1988).

Recently, several authors have demonstrated the presence of trophic factors delivered by specific systems. For example, Zhou and coworkers (Zhou, Averbach & Azmitia, 1987) described the enhanced proliferation of processes from raphe, but not locus coeruleus transplanted neurons, when they were placed in a serotonin-denervated hippocampus. Moreover, when an hippocampal transplant is placed near to an undamaged host hippocampus, the raphe neurons of the host are capable to innervate the new target sites, indicating that there are some kind of chemotaxis (Zhou, Averbach & Azmitia, 1988). So, it seems that some trophic factors could guide the neuronal processes with some specificity and finally could lead to the formation of new connections.

Therefore, in order to explain the establishment of connections between GN and VPM, there is the possibility that

this connections could be mediated by trophic factors (Zhou et al., 1987; Zhou et al., 1988). In our model, there are at least two potential sources of factors: the transplant by itself and the lesion-denervated host tissue (Cuningham, Haun & Chantler, 1987). The interaction of these factors could promote the connection of the transplant with the VPM and amygdala. One possible hypothesis to explain our behavioral results is based in the reconnectivity between VPM and GN, as proposed by Sharp and González (1986). These authors suggested that the re-connections between thalamus and cortex could stop the degenerative processes due to the lesion. Therefore, in this manner the graft could help to the restoration of the lost function.

In conclusion, we have demonstrated that after thirty days postgraft the animals with transplants were able to learn the aversive response in the CTA paradigm. The neurons in the transplant can express AChE in a time-dependent fashion. After 30 days the grafted neurons started to establish connections with the thalamus and amygdala of the host. Finally, the neurons in the older transplants showed a more mature morphology than those in the younger ones. All of these results suggest that morphological maturation and reconnectivity are necessary for recuperation of the acquisition of taste aversion learning in GN lesioned rats.

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	DAYS POSTRANSPLANT			
INJECTION SITE	15	30	45	60
THALAMUS	–	+	++	++
AMYGDALA	–	+	++	++

Table 1. Shows the qualitative analysis of the presence of the HRP labeled neurons inside the cortical grafts. – none, + scarce, ++ many HRP labeled cells, when compared with controls (see; Figure 2).

FIGURE LEGENDS

Figure 1. The graph illustrates the amount of saccharin consumed by 15, 30, 45 and 60 days postgraft groups. Left side shows the results from test trial prior to transplant. Right side shows the results of one test trial 15, 30, 45 and 60 days after transplant. * p 0.05 comparison with their own control groups. (Newmann-Keuls test).

Figure 2. Shows representative coronal section in dark field illumination, of a control subject in A. B and C shows HRP labeled neurons within homotopic grafts with 30 and 60 days after transplant respectively. A, B and C x 200.

Figure 3. Camera lucida drawings of Golgi-rapid impregnated neurons from 15, 30 and 60 day old transplants. In a: Drawing of Golgi-rapid impregnated neurons from 15 day old transplant. Some neurons show a round shaped soma and dendrites supporting sparsely spines (see, cells 2,3). Cell 1 shows more spines and cell 4 is a non spine neuron. b: Drawing from Golgi-impregnated neurons from 30 day old transplant. Neuron 1 shows a multipolar shape with spines on its dendrites. In c: Golgi-rapid impregnated neurons from 60 day old transplant. Neurons 1,2,3 show multipolar shape and their dendrites are covered with spines and in close relation with glial cells (a,b,c). Neurons 4 and 5 (from the border of the transplant) have large axons (A) and neuron 6 is a typical multipolar cell. Bars show a 100 microns at 400 x magnifications.

Figure 4. Shows the acetylcholinesterase reactivity of homotopic neocortical grafts, in 15 (A and B); 30 (C and D) and 60 (E and F) days postgraft. A, C and E x 4; B, D and F x 200.

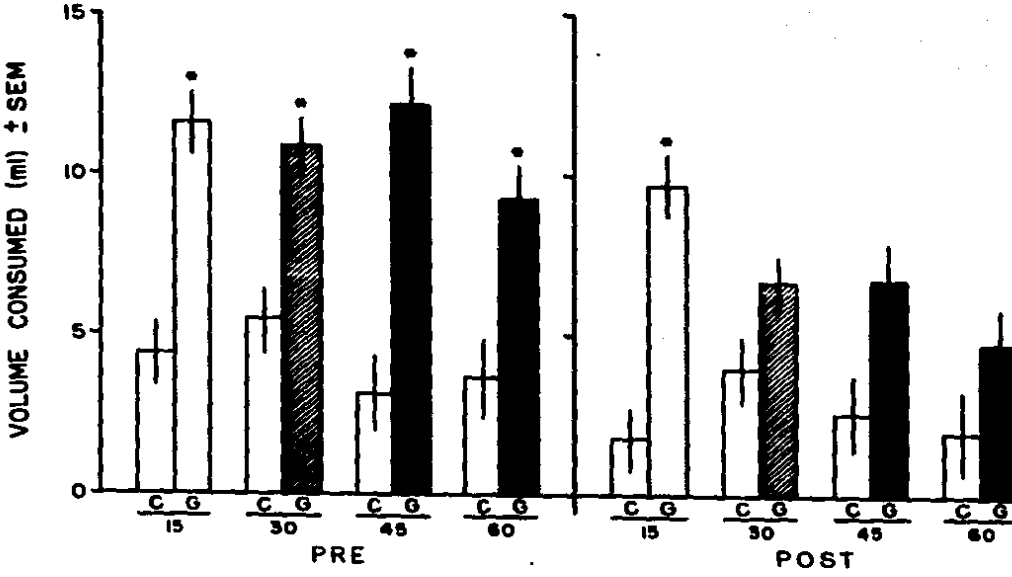

Fig. 1

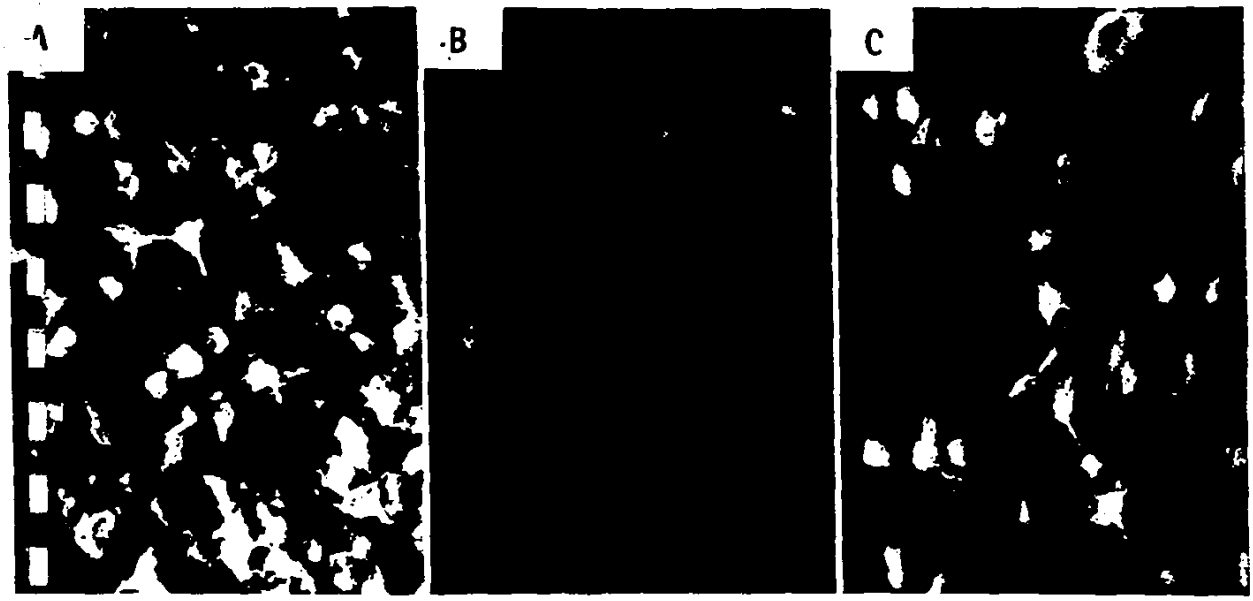

Fig. 2

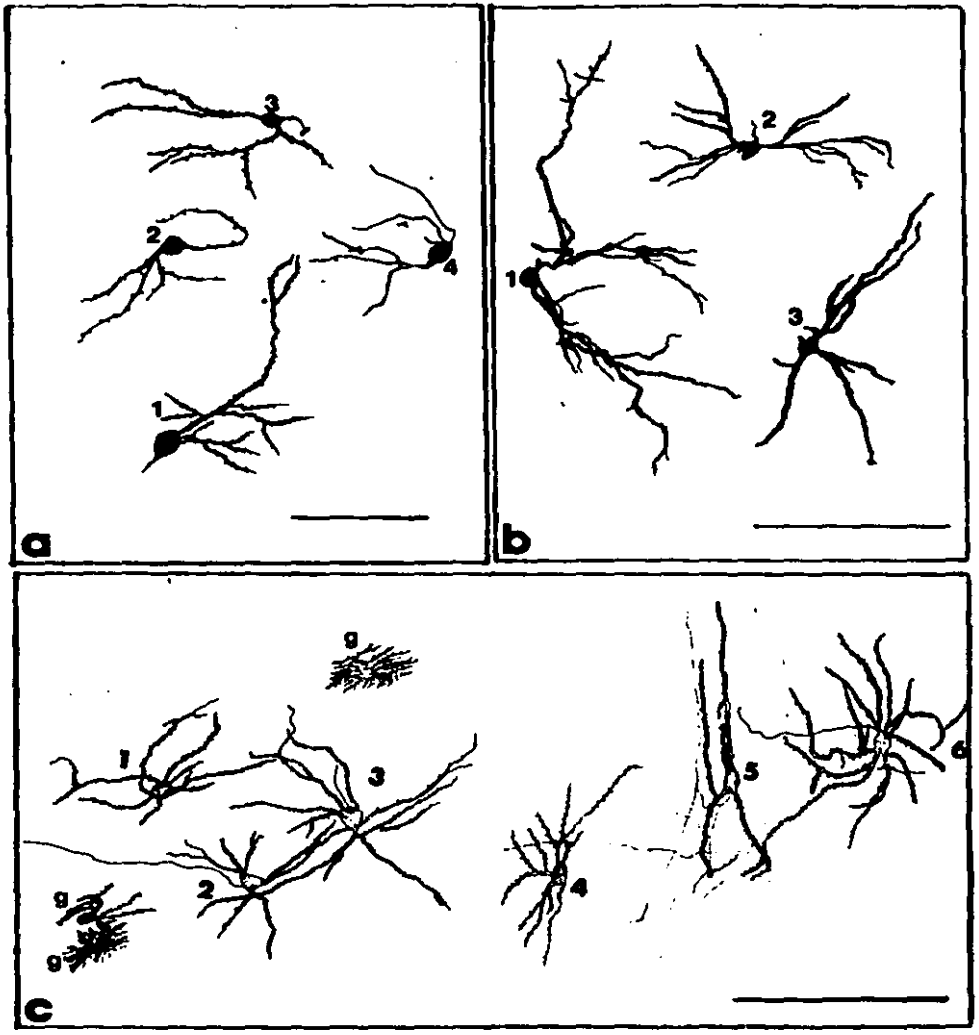

Fig. 3

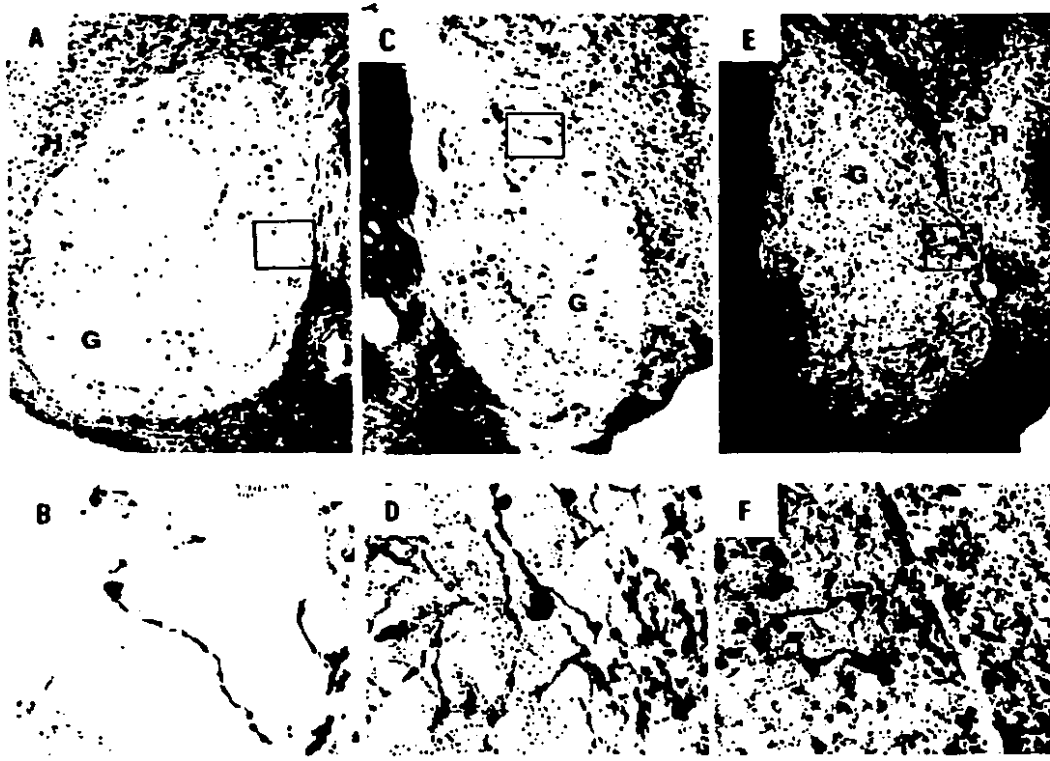

Fig. 4

TRABAJO IV

Preliminar

LA REINERVACION DE LOS TRANSPLANTES NEOCORTICALES DESDE LA PERSPECTIVA DEL FLUOROGOLD

Con el fin de observar la reconectividad de los transplantes neocorticales y confirmar así nuestros resultados con peroxidasa de rábano (HRP), decidimos efectuar algunos estudios empleando fluorogold, trazador axonal retrógrado fluorescente, que posee ventajas tales como: alta sensibilidad, no incorporación por fibras de paso, extenso marcaje de las proyecciones neuronales, amplio rango en el tiempo de sobrevivencia post-administración, administración circunscrita y compatibilidad con un gran número de técnicas histoquímicas.

Sujetos

Los sujetos empleados en este estudio fueron seis ratas machos de la variedad Wistar, con pesos entre 250 y 300g divididos de la siguiente manera:

-Grupo control I (n=2): Con administración bilateral de fluorogold en los núcleos VM y VPM talámicos (TAL)
-Grupo control II (n=2): Con administración bilateral de fluorogold en el complejo amigdalino (AMX)
-Grupo experimental (n=2): Con transplantes homotópicos en neocorteza gustativa (NG) y administración de fluorogold en complejo amigdalino y núcleos VM y VPM talámicos en el lado derecho e izquierdo de los sujetos respectivamente

Lesión y Transplante

Los sujetos experimentales fueron lesionados bilateral y electrolíticamente mediante técnicas estereotáxicas convencionales. Los electrodos fueron construídos con barras aisladas de acero inoxidable, excepto en la porción apical (0.5 mm aproximadamente) y conectados a un estimulador que permitió el paso de una corriente de 2 mAmp durante un minuto. Las coordenadas empleadas durante la cirugía de acuerdo al atlas estereotáxico de

Paxinos y Watson (1982) fueron las siguientes: NG (AP = + 1.2; L = ± 5; H = -5).Una semana después de practicada la lesión, los sujetos recibieron transplante bilateral de tejido neocortical procedente de fetos de 17 días de gestación, una vez seccionado el tejido neocortical correspondiente, se introdujo en una microjeringa Hamilton de 100 µm acoplada a un aparato estereotáxico, depositando así el tejido en el sitio correspondiente.

El tiempo de desarrollo post-transplante fue de 60 días, al cabo de los cuales se procedió a efectuar el análisis histoquímico.

Durante los procesos quirúrgicos de lesión y transplante los sujetos fueron anestesiados con pentobarbital (Nembutal) 50 mg/kg.

Histoquímica

Tanto los sujetos control como los experimentales recibieron aplicaciones de fluorogold en los sitios mencionados en la descripción de los grupos.

Los sujetos fueron anestesiados con hidrato de cloral al 10% (400 mg/kg), la administración se efectuó estereotáxicamente con una jeringa Hamilton de 1 µl en los siguientes volúmenes: 0.3 µl (en VM y VPM) y 0.5 µl (en AMX) de una solución al 2.5% de fluorogold y sol. salina fisiológica.

Cinco días después, todos los sujetos fueron perfundidos transcardialmente con solución salina fisiológica (200 ml p/rata) seguida de una solución de paraformaldehído al 4% y glutaraldehído al 0.1% (250 ml p/rata). Los cerebros fueron almacenados en una solución de sacarosa al 20% durante 48 horas, al cabo de las cuales fueron cortados (40 µm) y montados con solución de glicerol 1:2. Finalmente se procedió al análisis microscópico utilizando un microscopio de fluorescencia Nikon con un filtro de exitación UV - 323 nm y emisión a - 408 nm.

Resultados y Discusión Parcial

Los resultados mostraron que en el grupo control I con

administración en los núcleos talámicos (VM y VPM), se detectaron células marcadas con fluorogold en la neocorteza gustativa (Fig.1). por su parte en el grupo control II con administración en el complejo amigdalino, se detectaron células marcadas con fluorogold en la neocorteza gustativa y el tálamo (Fig.2). Finalmente en los sujetos experimentales, se detectó la presencia de células marcadas con el trazador en el interior de los transplantes, no habiendo fuertes diferencias entre los marcajes producidos por la administración en el complejo amigdalino o los núcleos talámicos (Fig.3). Sin embargo, la densidad de neuronas marcadas por el fluorogold fué considerablemente menor en comparación a la registrada en los grupos control, de manera similar a los resultados observados con HRP. la disminución, en cuanto a la densidad de neuronas marcadas, en el interior de los implantes, obedece a una serie de factores entre los que destacan el número de neuronas maduras dentro del transplante y en consecuencia, la densidad de la arborización terminal presente en la zona de administración (Jones (1975); Jaeger y Lund (1981) y Bjorklund y Stenevi (1984). Los sitios de administración fueron circunscritos ($1mm^3$ aproximadamente) y bien marcados, en tanto que el adecuado marcaje observado en los grupos control, confirma la elección apropiada del tiempo de sobrevida post-administración (5 días) seleccionado en nuestros experimentos a través de ensayo-error, considerando el lapso existente entre la administración del fluorogold, el inicio de la endocitosis, la tasa de transporte retrógrado así como la longitud de la vía estudiada.

Estos resultados apoyan ampliamente a nuestros resultados obtenidos a través del empleo de HRP, confirmando así la potencialidad de los transplantes homotópicos neocorticales de reinervar algunas de las zonas con las que la corteza intacta mantenía conexiones normalmente.

Fig. 1 Microfotografías de células marcadas con fluorogold en la neocorteza gustativa de ratas control. X 20 (Parte superior), X 200 (Parte inferior).

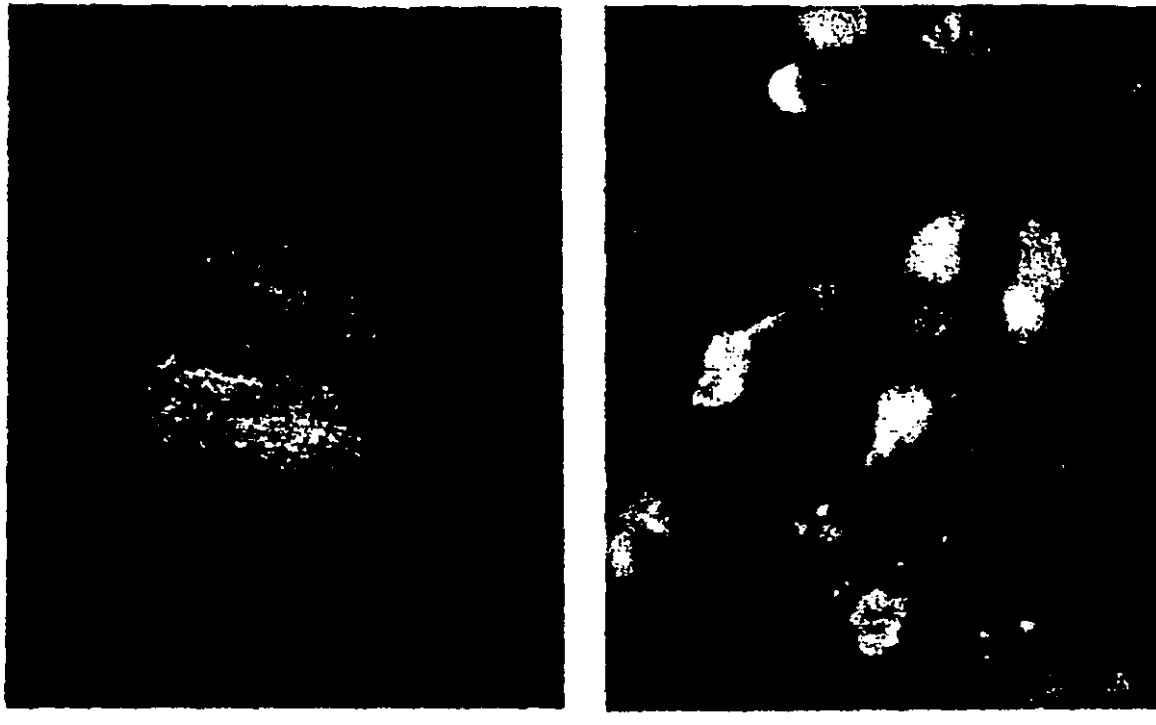

Fig. 2 Aspecto de los núcleos talámicos VM y VPM, tras la administración de fluorogold en el complejo amigdalino de ratas control. X 20 (Izquierda), X 200 (Derecha).

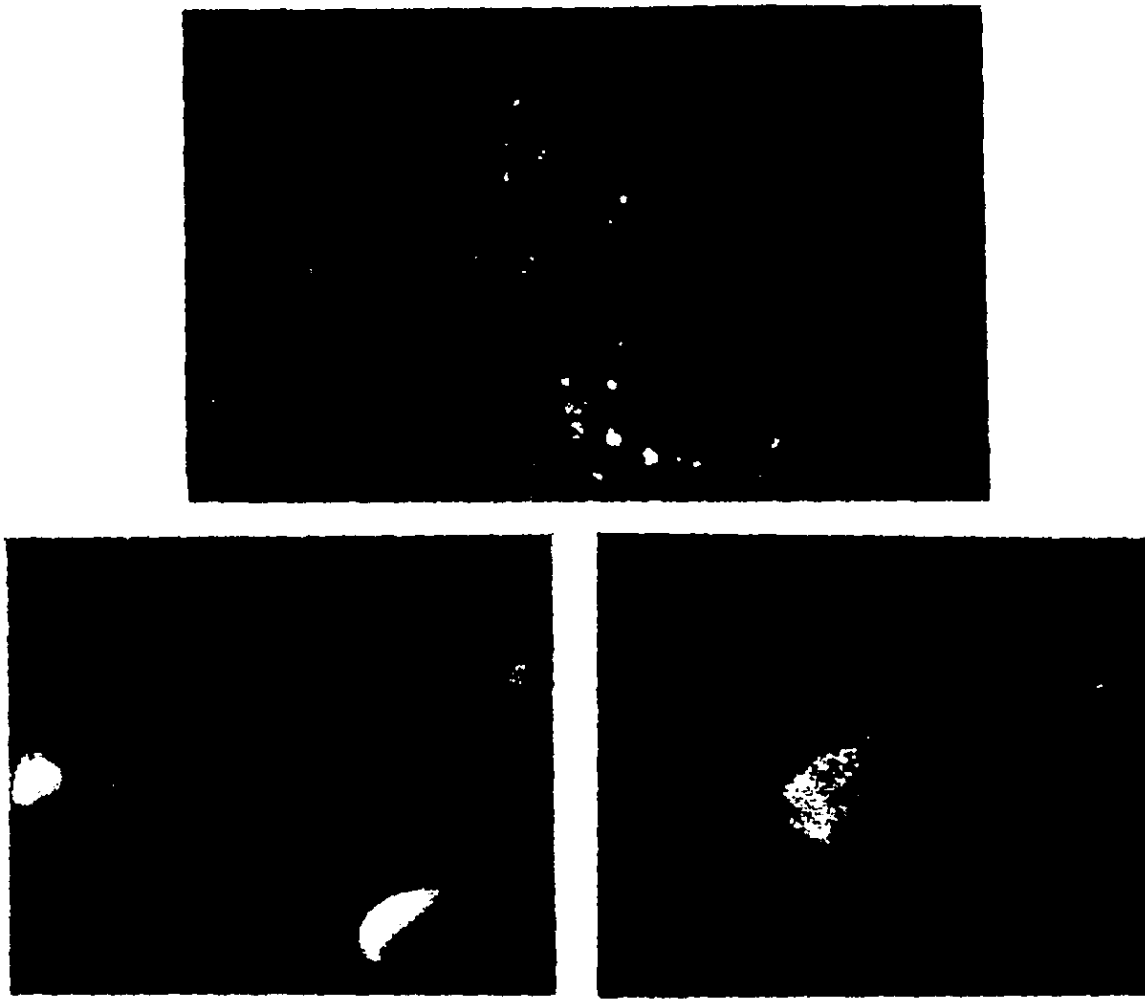

Fig. 3 Microfotografías de células marcadas con fluorogold, en el interior de transplantes homotópicos neocorticales. X 20 (Parte superior), X 200 (Parte inferior).

DISCUSION GENERAL

Una importante interrogante hoy por hoy en el ámbito de las neurociencias, es la relativa a la potencialidad plástica del SNC de los seres vivos, para responder ante una amplia gama de estímulos que forman parte del medio ambiente interno y externo de los organismos.

En el presente estudio, hemos tenido la oportunidad de abordar una de las expresiones de tal potencialidad, a través de la conjunción del modelo conductual del Condicionamiento Aversivo a los Sabores (CAS), y la técnica de los transplantes de tejido cerebral fetal.

Los resultados obtenidos en nuestros experimentos demuestran que los transplantes homotópicos fetales de neocorteza gustativa (NG), producen recuperación en la capacidad de adquirir el CAS, en ratas que previamente la habían perdido debido a la lesión de la corteza. Se demostró asimismo, que los transplantes homotópicos de NG, pero no los heterotópicos de tejido tectal, podían no solo restablecer las funciones cognitivas sino también la conectividad con el tálamo y la amígdala del tejido huésped, con quienes la NG mantiene conexiones normalmente. Estas observaciones subrayan la importancia de la especificidad de los tejidos transplantados en los procesos de integración.

A este respecto, Heusching y col. (1988), efectuaron un estudio en el que fue probada la actividad trófica de diferentes regiones del SNC (cortical, hipocampal, septal y estriatal) sobre neuronas embrionarias que fueron transplantadas o bien, mantenidas en cultivo, encontrando que mientras los transplantes de corteza e hipocampo se integraron y crecieron adecuadamente en las 4 regiones, los transplantes de septum, sufrieron atrofia al ser colocados en el estriado. Por su parte, los transplantes de estriado, se integraron y crecieron adecuadamente, tan solo cuando fueron colocados en la región estriatal. Los resultados obtenidos *in vitro*, respaldaron ampliamente estas observaciones. Los autores destacan el hecho de que en todos los casos los transplantes

homotópicos, mostraron una mejor integración, caracterizada por un alto y constante número de neuronas sobrevivientes.

Así Heusching y colaboradores, sostienen que cualquier área del cerebro adulto lesionada, presenta actividad trófica específica, preponderantemente dirigida hacia las células homotópicas embrionarias correspondientes. La habilidad para responder a la mencionada actividad, parece cambiar con el grado de afinidad entre el transplante y la zona receptora del huésped. La corteza, el hipocampo y el septum parecen actuar en el mismo sentido, mientras que el estriado despliega un patrón de respuesta radicalmente diferente. Por otro lado, se ha examinado (Gibbs *et al.*, 1986) el patrón de proyección y el grado de inervación, producidos por transplantes procedentes de 3 fuentes diferentes de células colinérgicas centrales. Los transplantes se colocaron en la formación hipocampal y los tejidos fuente fueron las regiones septal, habenular y estriatal. Los resultados revelan que el patrón de inervación es semejante en todos los casos, sugiriendo que éste se encuentra estrechamente correlacionado con el tipo de neurotransmisor. En la formación hipocampal, los transplantes de células colinérgicas (Lewis y Cotman, 1983) y monoaminérgicas (Bjorklund y Stenevi, 1979) emiten proyecciones con un arreglo característico, dependiente de su tipo de neurotransmisor, sugiriendo que el patrón de inervación es transmisor-dependiente. En 1987, Chanaud y Das transplantaron tejidos embrionarios neocorticales y de tallo cerebral, y observaron agudas diferencias en el grado de crecimiento alcanzado por los transplantes (mayor para los tejidos corticales y ostensiblemente menor para los tejidos procedentes del tallo cerebral). Estos hallazgos confirmaron las observaciones previas de los autores, en el sentido de que el crecimiento de un transplante neural está determinado en gran medida por su potencial de crecimiento, el cual a su vez está relacionado con la naturaleza de las células implantadas.

Algunos otros trabajos que destacan la importancia de la especificidad en la integración transplante-huésped, son los de

Whittemore *et al.*, (1985) y Gibbs y Cotman (1987). En ambos se enfatiza la influencia de la especificidad, de los factores liberados tras la lesión de alguna región del SNC, sobre la sobrevivencia y adecuada integración de los tejidos transplantados. Estos hallazgos sugieren que factores ambientales, inducidos específicamente por la destrucción de fibras homólogas del huésped, son los responsables de las diferencias en la sobrevivencia y la conectividad observadas en los transplantes (Gibbs y Cotman, 1987).

En otra etapa del presente trabajo, efectuada con la finalidad de comprender los procesos temporales que subyacen a la recuperación funcional y anatómica observadas, llevamos a cabo un análisis conductual y citoarquitectónico siguiendo el curso temporal (15, 30, 45 y 60 días) de desarrollo de los transplantes.Pudimos mostrar que la recuperación conductual comienza a manifestarse a partir de los treinta días de desarrollo post-transplante al tiempo que aparecen los primeros indicios de reconectividad, vascularización y maduréz estructural, alcanzando su mejor expresión hacia los 60 días. Durante los primeros 15 días de desarrollo post-transplante, los sujetos no mostraron ningún indicio de recuperación en el paradigma del CAS, en tanto que las observaciones histológicas mostraron la presencia de neuronas poco desarrolladas, con escasas proyecciones y revascularización incipiente.

El análisis de los resultados obtenidos con las técnicas de Golgi e impregnación argéntica, reveló la presencia de un proceso gradual de desarrollo, caracterizado por una reorganización neuronal, tanto en el tejido huésped como en el transplante, con una mayor densidad neuronal en el tejido transplantado.

En este sentido, podemos afirmar que los transplantes neocorticales fetales pueden adherirse al tejido huésped, presentando las siguientes características: 1) abundante vascularización; 2) una gran proliferación de células gliales en el borde de los implantes y ; 3) considerable proliferación de

fibras que atraviezan la interfase. Esto evidencia un proceso dinámico de interacción morfológica y funcional entre el transplante y el tejido huésped.

Sin embargo, los transplantes homotópicos neocorticales, no mostraron el característico arreglo laminar de la corteza intacta, lo cual concuerda con las observaciones de Mufson *et al.* (1987), quienes encuentran recuperación funcional e integración anatómica de transplantes fetales neocorticales, en ausencia del arreglo laminar correspondiente.

Otro aspecto abordado en el presente trabajo, fue el relativo a la detección de la presencia de algunos neurotransmisores en los transplantes neocorticales, y la posible participación de los mismos en los procesos de recuperación funcional. Estos estudios mostraron que los transplantes homotópicos neocorticales, que promueven la recuperación del aprendizaje en el CAS, liberan ACh, en tanto que en los transplantes heterotópicos (corteza occipital), que no inducen recuperación funcional, no se registra liberación del citado neurotransmisor. Esto sugiere una participación colinérgica en la recuperación conductual mediada por transplantes dentro del CAS (López, *et al.* en revisión).

Aunados a estas observaciones, los trabajos efectuados por Woolf y Butcher (1982) y Bermúdez-Rattoni *et al.* (1983), señalan que la acetilcolina juega un papel importante en el CAS. Existen evidencias en el sentido de que la neocorteza gustativa presenta considerable actividad colinérgica (acetilcolintransferasa CAT y acetilcolinesterasa AChE), así como de que el bloqueo farmacológico de la transmisión colinérgica provoca perturbaciones en la adquisición del CAS.

A este respecto, es importante considerar que el desarrollo de los neurotransmisores, forma parte de una serie de eventos involucrados en el establecimiento de la compleja circuitería de la corteza cerebral de los mamíferos. Existen reportes en el sentido de que algunas poblaciones celulares de la corteza en desarrollo, presentan lapsos en los cuales se expresan

transitoriamente algunos neurotransmisores (Luskin y Shats, 1985; Valverde y Facal-Valverde, 1987; Robertson, 1987). La aparición y desaparición de tales mensajeros químicos, puede estar orquestando el establecimiento de la circuitería cortical. Los neurotransmisores pueden jugar un papel importante en la organización de la corteza en desarrollo, así como en los procesos de integración transplante-huésped (Parnavelas y Cavanagh, 1988; Lipton y Kater, 1989). Por ejemplo, en el gato ha sido claramente demostrado (Luskin y Shatz, 1985; Valverde y Facal-Valverde, 1987), que las neuronas que se localizan por debajo de la capa IV de la corteza así como en la zona marginal, no sobreviven a la edad adulta y su desaparición coincide con la invasión de la placa cortical por las proyecciones callosas y tálamo-corticales, de manera que estas células generadas tempranamente en la subplaca, pueden fungir como blancos temporales o guías, para los axones talámicos y callosos (Chun *et al.*, 1987; Shatz y Luskin, 1986). D'Amato *et al.* en 1987, reportaron la presencia de zonas con inervación serotoninérgica densamente marcadas, en las áreas sensoriales primarias de la neocorteza de la rata, durante las primeras tres semanas de vida postnatal. Las zonas se ubican en las capas IV y VI, que son las principales áreas receptoras de las entradas talámicas. Estas zonas serotoninérgicas pueden influenciar el crecimiento de las proyecciones talámicas. Bear y colegas (1985) describieron un decremento transitorio de la población de fibras positivas a colinesterasa, en las capas IV y VI de la corteza visual de gato entre la cuarta y octava semanas de desarrollo post-natal. Esta observación es consistente con la noción de que las proyecciones colinérgicas, influencian la formación de conexiones durante el período crítico. Por último, en relación a este rubro, mencionemos que en 1987, Robertson demostró la expresión transitoria de acetilcolinesterasa en el sistema tálamo cortical en desarrollo. Tal expresión transitoria, tiene una duración de tres semanas al cabo de las cuales declina hasta alcanzar los niveles normales del adulto, al tiempo que las conexiones tálamo-corticales han sido bien establecidas.

Nuestros resultados señalan reiteradamente, la importancia de la integración anatómica de los transplantes y de la reconexión de los mismos, en los procesos de recuperación funcional. El número de estudios que demuestran la existencia de reconectividad por parte de los transplantes corticales, ha crecido considerablemente en los últimos años (Ebner y Erzurumlu, 1985; Wiegand y Gash, 1985; Gibbs *et al.*, 1986).

Recientemente, varios autores han demostrado la presencia de proyecciones procedentes de transplantes, tras la denervación específica de las zonas transplantadas. Tal es el caso de Zhou y colaboradores (1987), quienes describieron la creciente proliferación de proyecciones provenientes de transplantes de rafé, pero no de transplantes de locus coeruleus, cuando estos fueron colocados en el hipocampo denervado de sus proyecciones serotoninérgicas. Estas observaciones parecen indicar que algunos factores tróficos pueden guiar a las proyecciones neuronales con cierto grado de especificidad, permitiendo la formación de nuevas conexiones. Por tanto, con el fin de explicar el establecimiento de conexiones entre la NG y la amígdala y los núcleos talámicos VPM y VM, existe la posibilidad de que estas conexiones puedan ser mediadas a través de factores tróficos. En nuestro modelo existen al menos dos fuentes potenciales de factores: el transplante *per se* y el tejido huésped lesionado (Cuningham *et al.*, 1987). Ejemplos de la influencia de las lesiones, sobre los procesos de reconexión transplante-huésped pueden apreciarse en trabajos como los de Gibs y Cotman (1987) o los de Homann y Ebner en 1988, quienes realizaron un estudio en el que compararon transplantes embrionarios corticales colocados en huéspedes adultos normales, con transplantes similares, colocados en huéspedes adultos a los que previamente se les había lesionado el cerebro basal anterior (NBM), quedando eliminadas en consecuencia, la mayoría de las entradas colinérgicas corticales. Los resultados mostraron que la penetración de las proyecciones talámicas del huésped en los transplantes corticales, fue incrementada significativamente por

la lesión previa del NBM.

No se descartó la posibilidad de que tal lesión destruyera algunas vías no colinérgicas (serotoninérgicas y catecolaminérgicas) o bien que promoviera la liberación de factores tróficos, que coadyuvaran al establecimiento de tales conexiones.

Algunos otros estudios que muestran la inducción de rebrote axonal (sprouting), como respuesta ante la deplesión colinérgica, son los de Crutcher, (1982) y Gage (1984), en los que se ha mostrado que lesiones tanto de septum como de cerebro anterior basal, originan rebrote de fibras simpáticas en sus respectivas áreas blanco. Los mecanismos propuestos para tratar de explicar la participación de la ACh en los procesos de reinervación, señalan que la inervación colinérgica inhibe activamente la habilidad de elongación de las fibras respectivas, a través de efectos mediados por receptores colinérgicos, o bien, que este neurotransmisor induce la producción de respuestas tales como la formación de sinapsis, estabilizando y por tanto impidiendo la elongación de las fibras correspondientes.

Una posible hipótesis para explicar nuestros resultados conductuales, se basa en la reconectividad entre la corteza y los núcleos talámicos, de manera similar a lo propuesto por Sharp y colaboradores (1986), quienes sugieren que la reconexión entre el tálamo y la corteza, puede detener el proceso degerativo originado por la lesión. En consecuencia, los transplantes pueden contribuir en este sentido, al restablecimiento de la función perdida.

En conclusión, nuestros estudios han demostrado que:

1) La lesión de la NG provoca fallas en la adquisición del CAS, que no son reversibles por sí solas, al menos por periodos de seis meses
2) Los transplantes homotópicos neocorticales de tejido fetal, producen recuperación en la capacidad de adquirir el CAS

3) Los transplantes homotópicos neocorticales, establecen conexiones con la amígdala y el tálamo (núcleos VM y VPM) del tejido huésped, con los cuales la corteza gustativa mantiene conexiones normalmente

4) Los transplantes heterotópicos de tejido fetal tectal, no mostraron indicios de recuperación funcional, ni de integración anatómica

5) La recuperación funcional de los transplantes homotópicos neocorticales, comienza a manifestarse hacia los 30 días de desarrollo post-transplante

6) Tras 30 días de desarrollo post-transplante, las neuronas transplantadas comienzan a establecer conexiones con el tálamo y la amígdala del tejido huésped, al tiempo que aparecen los primeros indicios de vascularización y maduréz estructural

7) Los transplantes homotópicos neocorticales liberan ACh, mientras que en los transplantes heterotópicos de corteza occipital que no inducen recuperación funcional, no se registra liberación del citado neurotransmisor.

8) Las neuronas de los transplantes homotópicos, expresan AChE de manera tiempo-dependiente

9) Las neuronas de los transplantes con 60 días de desarrollo, presentan una morfología madura, comparada con los transplantes de 15, 30 y 45 días

10) La mejor expresión de la recuperación funcional en los sujetos con transplantes homotópicos neocorticales, se presenta también hacia los 60 días post-transplante

Nuestros resultados sugieren que la maduréz morfológica y la reconectividad entre el transplante y el huésped, son necesarias para la adecuada expresión de la recuperación conductual del CAS, en sujetos previamente lesionados en la neocorteza gustativa.

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