UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO

EVALUACION HISTOLOGICA, BIOQUIMICA Y CONDUCTUAL EN RATAS CON TRANSPLANTES CORTICALES DE TEJIDO NERVIOSO FETAL

TESIS QUE PARA OBTENER EL GRADO DE

MAESTRO EN INVESTIGACION BIOMEDICA BASICA

PRESENTA EL

LIBB. JUAN FERNANDEZ RUIZ

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INDICE

INTRODUCCION

A partir de la década de 1970, se ha generalizado la aceptación de la técnica de transplantes de tejido nervioso como una herramienta para el estudio del cerebro. Ya con anterioridad se habían realizado algunos intentos de transplantes, sin embargo no existió mayor interés en el tema, por lo que antes de 1970 en la literatura sólo se pueden encontrar algunas comunicaciones esporádicas (Bjorklünd and Stenevi 1985).

Es hasta los primeros reportes de Das y Altman (1971,1972) que empieza a despertarse un interés común por el estudio de los transplantes. Así, a finales de los 70s y principios de los 80s, se empiezan a incluir los términos en inglés "transplant" y "graft" como palabras claves para la identificación de dichos trabajos. Tomando como referencia el índice de materias de las memorias de los congresos de la "Society for Neuroscience", podemos observar el creciente interés por este tema de investigación (fig. 1). En base a lo anterior, considero que estaría fuera de lugar en la presente tesis intentar discutir todos los campos en donde se ha aplicado esta poderosa herramienta, por lo que mencionaré los modelos experimentales más estudiados (tabla 1) y profundizaré únicamente en aquellos que conciernan a la relación transplante-aprendizaje y memoria.

TRANSPLANTES Y APRENDIZAJE Y MEMORIA

La corteza cerebral y el sistema límbico, son dos de las regiones del Sistema Nervioso Central (SNC) que han sido más

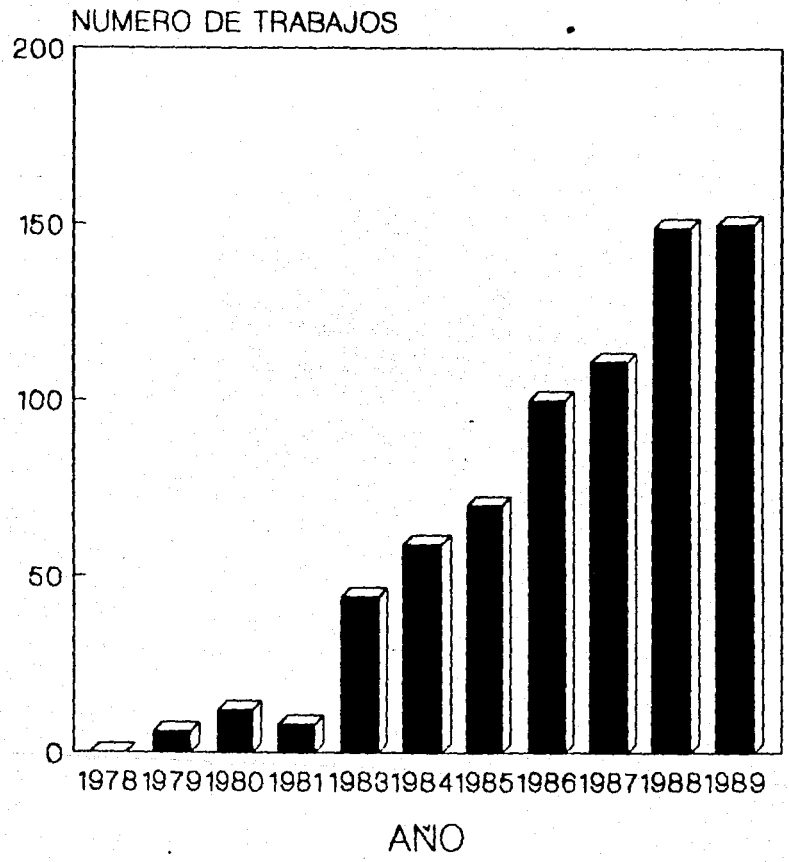

FIGURA 1. NUMERO DE TRABAJOS PUBLICADOS BAJO EL TEMA DE TRANSPLANTES O GRAFTS EN LOS CONGRESOS DE LA SOC. FOR NEUROSC.

relacionadas en los procesos de aprendizaje y memoria; dentro del sistema límbico, la formación hipocampal ha recibido especial atención, por lo que a continuación describiré algunos de los estudios más relevantes que conciernen a estas estructuras -formación hipocampal y corteza cerebral- y a los transplantes cerebrales.

ENFERMEDAD DE PARKINSON	LESION DE LA VIA NIGRO-ESTRIATAL	TRANSPLANTE DE CELULAS CROMAFINES O DE S.N. FETAL
ENFERMEDAD DE HUNTINGTON	LESION DEL NUCLEO CAUDADO	TRANSPLANTE DE CELULAS DE NUCLEO CAUDADO FETAL
ENFERMEDAD DE ALZHEIMER	LESION DEL NBM Y FORMACION HIPOCAMPAL	TRANSPLANTE DE CELULAS DEL NBM O FORMACION HIPOCAMPAL
ISQUEMIA	ISQUEMIA EXPERIMENTAL	TRANSPLANTE DE CELULAS DE LA FORMACION HIPOCAMPAL.
RETINOPATIA	RETINOPATIA EXPERIMENTAL	TRANSPLANTE DE CELULAS DE LA RETINA.

TABLA1.PRINCIPALES MODELOS EXPERIMENTALES DONDE SE HAN REALIZADO EXPERIMENTOS EMPLEANDO LA TECNICA DE TRANSPLANTES EN EL SISTEMA NERVIOSO. PARA UNA REVISION GENERAL, VER:PROGRESS IN BRAIN RES. V.78

TRANSPLANTES EN LA FORMACION HIPOCAMPAL

La formación hipocampal se encuentra formada por diversas estructuras como son el hipocampo, el giro dentado, la corteza entorrinal y el subículo (Swanson, 1983).

El hipocampo es una de las estructuras más estudiadas del S.N.C., lo cual es comprensible ya que posee 3 características que la hacen muy atractiva para la investigación. Primero, su estructura neuroanatómica es laminada, y recibe sus aferencias mas importantes en campos específicos de su anatomía; además, estas conexiones están claramente establecidas. Segundo, ha sido relacionada con algunos modelos experimentales de aprendizaje y memoria. Finalmente, es una de las pocas regiones del SNC en donde se han podido demostrar diferentes tipos de plasticidad neuronal (Swanson, 1983).

Esta estructura no ha sido ajena a los estudiosos de los transplantes de sistema nervioso, por el contrario, las 3 características ya mencionadas la hacen uno de los principales focos de experimentación en este campo. Es en la formación hipocampal, al igual que en la vía nigro-estriatal, donde se han llevado a cabo los estudios más sistemáticos, tanto en la anatomía como en la fisiología de los transplantes.

Se han realizado diferentes experimentos neuroanatómicos para estudiar la inervación del hipocampo por los transplantes. Por ejemplo la conexión corteza entorrinal-hipocamo corre por la vía perforante y es una de las principales vías de acceso de

informacion con las que cuenta el hipocampo. En una serie de experimentos, Cotman y cols. (1988) y Gibbs y Cotman (1987), investigaron la relación neuroanatómica entre estas dos estructuras mediante el implante de corteza entorrinal fetal cerca del hipocampo. Encontraron que el máximo crecimiento e innervación de los transplantes de corteza entorrinal al hipocampo hospedero se dió cuando se lesionó previamente la corteza entorrinal nativa, pero no cuando se lesionó corteza occipital o area septal. Estos autores (Cotman y cols., 1988) inclusive demuestran que los transplantes de corteza entorrinal son capaces de inducir recuperación conductual en una prueba de memoria espacial, seis meses depués de haber lesionado a los sujetos (Gibbs y cols. 1987).

Un modelo diferente en el que se estudian las aferencias a la formación hipocampal, analizan las conexiónes del área septal con el hipocampo; esta vía corre por la fimbria-fórnix y es de fácil acceso para el investigador. Las lesiones de esta vía también han sido utilizadas para observar el desarrollo de transplantes y su posible inervación al hipocampo. En sujetos con lesiones en la fimbria-fornix, se ha observado que los transplantes de área septal mandan axones colinérgicos únicamente a los campos colinérgicos terminales que normalmente inervan, aunque la lesión del la fimbria-fornix deja también otras zonas denervadas del hipocampo. Cabe recordar que lesiones de la corteza entorrinal no afectan la supervivencia de los transplantes de area septal, pero si incrementan la de los transplantes de corteza entorrinal;

de igual manera la lesión de la fimbria-fornix no repercute en el transplante de corteza entorrinal, pero si afecta a los transplantes de área septal (Kromer 1985; Gage y Björklund 1986; Gage y cols. 1985).

En 1982 Low y cols. demostraron que animales con transplante de área septal previamente lesionados en la fimbria-fornix, pero no los unicamente lesionados, incrementaron su número de aciertos en un laberinto de 8 brazos cuando se les aplicó un inhibidor de la acetilcolinesterasa (fisostigmina) (Low y cols. 1982). De igual manera, Dunnet y col (1982) demostraron que animales con transplantes de área septal previamente lesionados en la fimbria-fornix, aprendieron a alternar en un laberinto de "T" con la misma eficiencia que el control; no así los animales con transplante de locus coeruleus o simplemente lesionados en la fimbria-fornix. Además en este último estudio, correlacionaron la recuperación conductual con el grado de inervación colinérgica del transplante al hipocampo hospedero (Dunnet y cols. 1982).

Finalmente, cabe mencionar aquellos estudios donde existe una lesión en sí del hipocampo. Woodruff y col (1987) transplantaron hipocampo o tallo cerebral fetal en cavidades hechas previamente en el hipocampo del hospedero. Sus resultados indicaron que ambos tipos de transplantes crecieron y fueron inervados por el hospedero. Sin embargo, solo observaron la recuperación conductual en una tarea de aprendizaje en el grupo de ratas que recibió transplante de hipocampo. Resultados similares se han obtenido en otros modelos de lesión del hipocampo, en los cuales

el transplante induce una recuperación conductual medida en el aprendizaje de un laberinto radial de 8 brazos (Tandon y cols. 1988) o en otro tipo de laberintos (Kimble y cols. 1986).

TRANSPLANTES EN LA NEOCORTEZA

Los estudios realizados en esta estructura siguen generalmente dos aproximaciones. a) Lesión de la neocorteza en si; y b) Lesión de sus conexiones, como por ejemplo las aferencias que recibe del núcleo basalis magnocelularis .

A.- Lesion de la corteza cerebral.

Los primeros estudios conductuales con transplante de corteza fueron realizadas por Labbe y cols. (1983), en los cuales lesionaron la corteza frontal y midieron la tarea de laberinto de "T" con alternancia forzada. En dichos estudios encontraron que las ratas lesionadas ejecutaron la tarea al azar, mientras que aquellas ratas que recibieron el transplante de corteza, mejoraron significativamente su ejecución. Cabe mencionar que las pruebas conductuales fueron llevadas a cabo poco tiempo después de haber realizado el transplante, por lo que los autores consideran que es difícil que se haya establecido algún tipo de reconstrucción de las vías o circuitos involucrados en dicha conducta (Labbe y Col. 1983).

En 1986, Kesslak y cols. encontraron de manera sorprendente que transplantes de astrocitos purificados, aceleraban la recuperación conductual en una prueba de laberinto de "T" de alternancia forzada, de manera igual a la tasa de aprendizaje de los controles; estableciéndose así que no era necesario en esta

caso, la presencia neuronal para el restablecimiento de la realización de una tarea de aprendizaje (Kesslak y cols. 1986).

Sin embargo, otros grupos si han encontrado el establecimiento de conexiones hospedero-transplante cortical, como son aquellos estudios realizados en el modelo de la vía tálamo-cortical. Por ejemplo, en los transplantes realizados en la corteza occipital, se ha observado el restablecimiento de la vía núcleo geniculado lateral (NGL)-corteza occipital, aunque no se ha estudiado en este caso algún modelo conductual. Otra aproximación en este sentido es la comunicada por Isacson y col (1988), donde encuentran el establecimiento de conexiones del transplante cortical con el núcleo basalis magnocelularis así como con el locus coeruleus, en ratas previamente lesionadas con ácido kaínico o con el ácido N-metil-D-Aspártico. Además demuestran la actividad de la GAD, la ChAT y la captura de H^3-Glutámico dentro de los transplantes. Basados en esos datos, los autores postulan la existencia de conexiones y la funcionalidad metabólica de los transplantes (Isacson y cols. 1988).

Finalmente, los estudios más aproximados a un modelo de integración funcional de un transplante neuronal, son los trabajos publicados por el grupo de F.F.Ebner (1988,1989), donde se observa la innervación funcional del tálamo al transplante de corteza sensorimotora. El procedimiento que siguieron se dividió en cuatro fases: a) Sección de la rama infraorbital del nervio trigémino. b) Lesión cortical seguida inmediatamente por el implante de tejido embrionario neocortical. c) Registro unitario

de las neuronas del transplante y de la corteza hospedera. 4) Análisis del crecimiento de las fibras tálamo-corticales dentro del transplante, mediante el uso de peroxidasa de rábano. Estos investigadores reportaron que en los sujetos con lesión del nervio trigémino el 22% de neuronas registradas dentro del transplante, responden a la estimulación del nervio trigémino, o del movimiento de las vibrisas; encontrandose el 85% en los sujetos normales. Además encontraron la presencia de peroxidasa de rábano en el transplante, con lo cual demostraron el establecimiento de conexiones entre el transplante cortical y el tálamo del hospedero (Ebner 1988; Ebner y cols. 1989).

De igual manera, Sorensen y cols. (1989) y Neafsey y cols. (1989), demostraron el establecimiento de conexiones corteza-tálamo y lo que es más importante, demostraron también el establecimiento de una relación funcional en este mismo modelo. Dichos autores probaron que transplantes de corteza sensorimotora en una rata hospedera previamente lesionada, respondieron tanto a la estimulación talámica como a la estimulación eléctrica de las patas delanteras (Sorensen y cols. 1989; Neafsey y cols. 1989).

B.-Lesión de las conexiones corticales.

Con respecto a esta segunda metodología experimental, el modelo que se pretende seguir está basado principalmente en los hallazgos que se han obtenido en el estudio de la enfermedad de Alzheimer y el envejecimiento. En dichos trabajos se ha reportado el decaimiento de los niveles de noradrenalina (Zornetzen and Thompson 1982) y acetilcolina (Coyle y cols. 1983)

entre otros neurotransmisores en la corteza cerebral. Además se ha observado degeneración de la corteza en si, al igual que en el hipocampo (Price, 1986).

Existen varios modelos experimentales que comparten algunas características con la enfermedad de Alzheimer, y que pueden ser de provecho para la investigación, como es el caso de los animales viejos que presenten deficiencias conductuales o motoras medibles experimentalmente (Price, 1986). Sin embargo por el alto costo de dichos animales, entre otras razones, se han ideado otros modelos experimentales que semejen a la enfermedad de Alzheimer, como es la lesion del núcleo basalis magnocelularis. En animales con lesión de este núcleo, existe una depleción de acetilcolina en la corteza cerebral, que semeja a la presentada por los pacientes de enfermedad de Alzheimer, además de que también presentan deficiencias en algunas pruebas conductuales (Smith, 1988; Gage y cols. 1985.; Dunnet y cols. 1985).

En 1985 A. Fine y cols. reportaron que transplantes de tejido del cerebro anterior fetal, mejoran la ejecución de una prueba de prevención pasiva en ratas con lesiones previas del núcleo basalis magnocelularis (Fine y cols. 1985). Aun más, en el modelo de ratas viejas Dunnet y cols. (1988) y Collier y cols. (1988) encontraron que transplantes colinérgicos y transplantes noradrenérgicos respectivamente, fueron capaces de mejorar tareas de aprendizaje en ratas viejas que habían mostrado deficiencias conductuales previamente al transplante.

TRANSPLANTES DE TEJIDO NERVIOSO: CONCLUSION.

A lo largo de esta introducción hemos visto algunos de los diferentes modelos donde se ha empleado la técnica del transplante de tejido nervioso, habiendo enfatizado los modelos principales donde se ha estudiado la relación transplante-aprendizaje. De lo anterior es posible obtener algunas pautas generales que se han concluído respecto a los transplantes de tejido nervioso.

1.- Los transplantes de tejido nervioso son capaces de sobrevivir en un hospedero de su misma especie.

2.- Los transplantes de tejido nervioso son capaces de expresar marcadores fisiológicos normales que reflejan su funcionamiento dentro del hospedero.

3.- Los transplantes de tejido nervioso son capaces de establecer conexiones con el hospedero.

4.- Finalmente, los transplantes de tejido nervioso son capaces de inducir recuperación funcional de conductas previamente perdidas por el hospedero, inclusive tareas de aprendizaje (Bermúdez-Rattoni y cols., 1987; Escobary cols. 1989; Fernández- Ruiz y cols.), o mas aun, de conductas innatas (Paredes y cols. 1989). Cabe mencionar que hasta la fecha en que se redactó la presente tesis, unicamente existian dos reportes que se refieren a la recuperacion del condicionamiento aversivo a los sabores -ver mas adelante- debido al transplante de tejido nervioso, el primero de ellos es de nuestro grupo de trabajo (Bermúdez-Rattoni y cols., 1987), y el segundo fué desarrollado por Yirmiya y cols. (1988); ambos reportes concuerdan en el efecto positivo del transplante sobre la expresion conductual.

EL CONDICIONAMIENTO AVERSIVO A LOS SABORES

Una condicion esencial para la supervivencia de cualquier organismo es su capacidad para obtener comida suficiente y de una composición adecuada. Los animales son capaces de seleccionar de entre toda una gama de alimentos, aquellos que les proporcionen una dieta balanceada de minerales, vitaminas, carbohidratos y aminoácidos esenciales. Lo anterior ha sido demostrado en experimentos de selección de alimentos, que han indicado que mediante procesos de aprendizaje, se relaciona la información de las propiedades sensoriales de la comida con las subsecuentes consecuencias de su ingestión (Richter, 1943; Domjan, 1977). Por otro lado se ha demostrado que la expresión de conductas innatas y adquiridas permiten a los organismos reconocer no solo a los alimentos nutritivos, sino que también a los que les son tóxicos (Grill y Norgren, 1978). Una de dichas conductas, es la evaluación anticipatoria de las probables consecuencias de la ingestión de un alimento en particular; las dietas familiares son aceptadas o rechazadas de acuerdo con la experiencia previa del sujeto (Domjan 1975, 1977).

Según García y Koelling (1966), para que la comida que es por primera vez degustada sea evaluada, se requiere que sus propiedades sensoriales (generalmente el gusto) sean conservadas en un "archivo mnemonico" de corto plazo, hasta que se defina su valor nutritivo o toxico mediante la información del funcionamiente del sistema gastrointesinal. Es decir, el valor

será positivo si se consumió, por ejemplo, glucosa; en cambio si el alimento resultó tóxico, dando lugar a síntomas de envenenamiento visceral, el valor sera negativo. El marcaje negativo de un alimento será manifestado subsecuentemente como una aversión al gusto, y por lo tanto, una disminución en el consumo de dicho alimento. Este fenómeno es conocido como el condicionamiento aversivo a los sabores (CAS) (Garcia y Koelling 1966).

Los modelos que se han desarrollado para el estudio de este fenómeno siguen generalmente una metodologia ya estandarizada. A sujetos privados de agua se les da acceso a este líquido durante 10 minutos tanto en la mañana, como en la tarde. Se cuantifica el consumo diario de agua de los sujetos durante 10 días, a este consumo de agua se le conoce como linea base de ingesta. Al día siguiente se les da acceso a la solución deseada, que puede ser una solución de cloruo de litio, el cual produce una severa irritación gástrica, o una solución de sacarina sódica, seguida media hora después de LiCl, para producir la irritación gástrica, a esto se le conoce como adquisición de la aversión. Los siguientes días se les da acceso al agua a los sujetos hasta que de nueva cuenta, alcanzan la linea base de ingesta. Una vez restaurada la linea base se les da acceso a NaCl (que para la rata es indistinguible del LiCl), o a la sacarina sódica, según sea el caso. Ha sido ampliamente demostrado que los sujetos sometidos a esta metodologia, desarrollan un condicionamiento aversivo al sabor que ha sido apareado a la irritación gástrica; esto se

demuestra porque dichos sujetos disminuyen notoriamente la ingestión de la solución ya sea de NaCl o de sacarina sódica. (Garcia y col., 1986).

Las vías neurales involucradas en este fenómeno comienzan en los receptores gustativos de la lengua, estos comunican su información vía los nervios VII y IX al núcleo del tracto solitario, el cual recibe tambien información de los receptores gustativos de la laringe y la faringe vía el nervio X. El núcleo del tracto solitario también recibe información visceral del nervio vago, el cual es sensible a la irritación del estomago; este núcleo también recibe eferentes que provienen del área postrema, región muy importante en la producción del vómito en las especies que lo presentan. El segundo relevo gustativo se encuentra en el área gutatoria del puente, en el complejo parabraquial; en esta zona se han encontrado neuronas que responden tanto a los estimulos gustativos, como a los viscerales. A partir de este núcleo, se encuentran proyecciones que van a estructuras del cerebro anterior como la amigdala, el hipotálamo o la substancia inominata (Norgren, 1977). Otro paquete de fibras asciende al talamo ventro medial y al ventro postero medial para de ahí ascender a la corteza gustativa, la cual está localizada en la rata justo por encima de la fisura rhinal, rodeando a la arteria cerebral media. (Norgren, 1977).

Finalmente cabe mencionar que lesiones en diferentes niveles de este circuito producen la pérdida del condicionamiento aversivo a los sabores; por ejemplo, en el laboratorio hemos demostrado que

lesiones a la amigdala, el núcleo ventro postero medial del tálamo, o la corteza gustativa producen dicha pérdida.

OBJETIVO

El primero de dos objetivos de la tesis de maestría fué determinar si el transplante establecía o no conexiones con el tálamo gustativo y con la amigdala del hospedero; regiones que normalmente forman conexiones con la corteza gustativa.

El segundo objetivo fué determinar el curso temporal tanto de las conexiones del transplante, como de la recuperación conductual de la expresión del CAS.

Con este panorama en mente, me permito presentar en esta tesis la linea principal de experimentos que realicé durante mis estudios de maestría. Básicamente el trabajo se basó en los estudios del efecto producido por el transplante de tejido nervioso fetal, en ratas previamente lesionadas en la corteza insular gustativa, y su repercusión en una conducta de aprendizaje medida en el modelo conocido como condicionamiento aversivo a los sabores.

La parte experimental de la tesis está dividida en 3 partes. La primera es un artículo a modo de revisión, que permite entender las generalidades del trabajo (Bermúdez-Rattoni y cols. 1989). El segundo articulo demuestra las conexiones transplante-hospedero y la especificidad de la recuperación inducida por los transplantes (Escobar y cols. 1989).

Finalmente, el tercer articulo demuestra la recuperación

conductual con respecto al tiempo y su relación con la conectividad transplante-hospedero así como la maduración celular del transplante en sí (Fernández-Ruiz y cols. 1989).

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

Federico Bermúdez-Rattoni, Juan Fernández and Martha Lilia Escobar

Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México, D.F.

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 electrophysiological 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 (García et al., 1982; 1985). This difference in conditioning has been termed cue-consequences specificity (García 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).

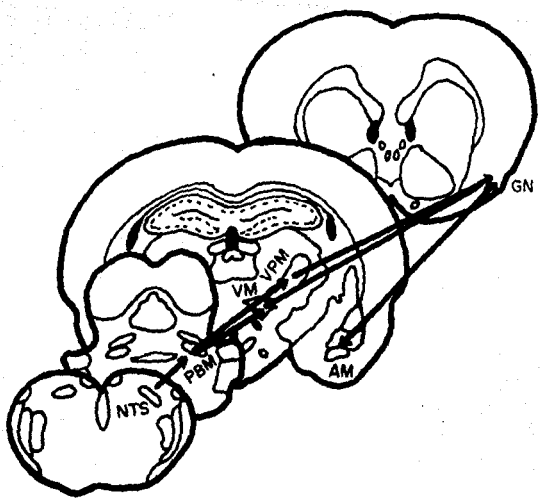

Fig. 1. Schematic drawing outlining the gustatory and visceral pathways in the rat. Abbreviations: (NTS) solitary nucleus, (PBM) medial parabrachial nucleus of the pons, (VM, VPM) ventromedial and posterior ventromedial thalamic nuclei, (AM) amygdala, (GN) gustatory neocortex.

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).

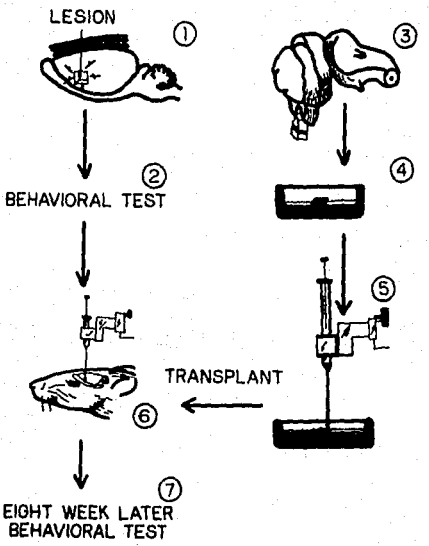

Fig. 2. Schematic representation of the methodological sequence. 1. Electrolytic lesions in the GN. 2. Behavioral test (see Fig. 3). 3, 4 and 5 transplant procedure; obtention of solid tissue from embryonic rats donnors; solid tissue block was put into a petry dish and aspirated by 100 ul Hamilton syringe. 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).

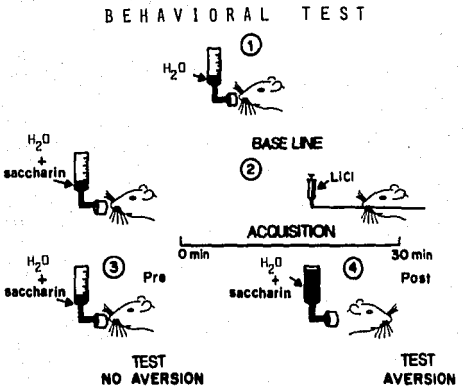

Fig. 3. Schematic representation of behavioral training sequence. 1. Training for 10 days to drink water for 5 minutes. 2. Acquisition day; presentation of saccharin disolved in the water (.1%) followed 30 min later with an infusion of LiCl (IG; 190 mg/kg). 3. Test day with saccharin in the water; Lesioned 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 CGN 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.

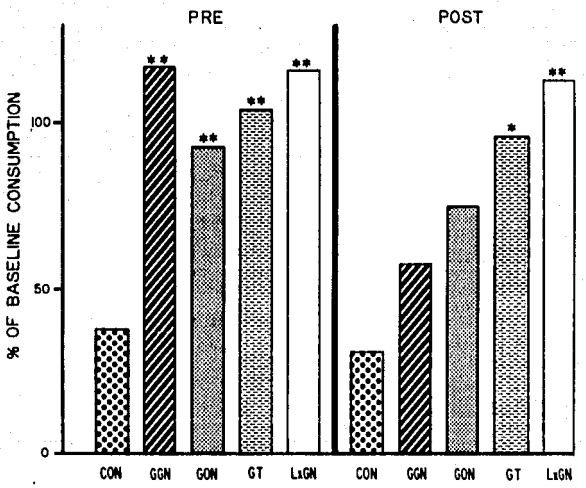

Fig. 4. The bars illustrate the amount of saccharin consumption by control, grafted and lesioned groups. Consumption is expressed as the percentage of each group's previous day water baseline, left side shows the results from one taste test trial prior to transplant. Right side shows the results of one 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).

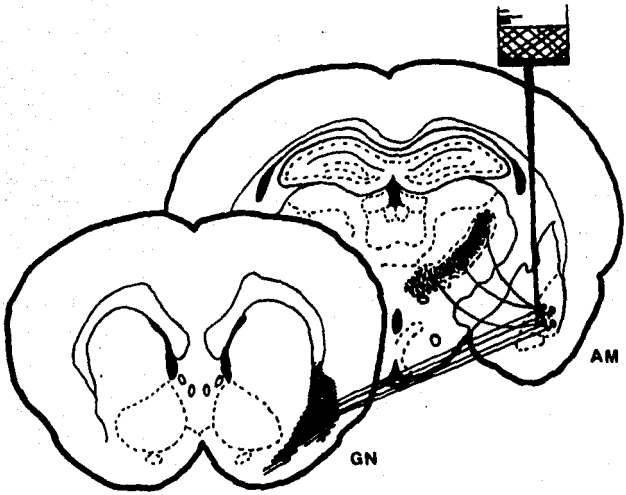

Fig. 5. Schematic representation of HRP injection in the amygdala (AM) and retrogradely labeled neurons within the gustatory neocortex (GN) and ventromedial nucleus of thalamus (VPM).

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

Martha Escobar[1], Juan Fernández[1], Rosalinda Guevara-Aguilar[2] and Federico Bermúdez-Rattoni[1]

[1]*Departamento de Neurociencias, Instituto de Fisiología Celular and* [2]*Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México D.F. (Mexico)*

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Key words: Conditioned taste aversion; Fetal brain transplant; Grafting; Horseradish peroxidase; Gustatory neocortex

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[3]. 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

Correspondence: Federico Bermúdez Rattoni, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apartado postal 70-600, 04510 México D.F., Mexico.

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 temporoparietal 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.

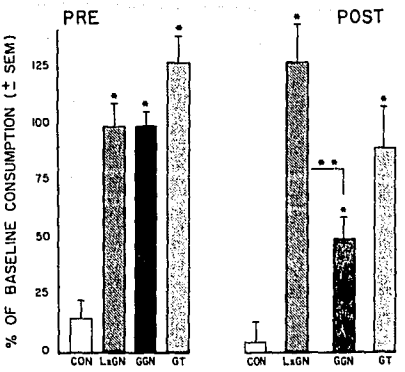

Fig. 1. The graph illustrates the amount of saccharin consumed by control, lesioned and grafted groups. Consumption is expressed as the percentage of each group's previous water baseline. Left side shows the results from one taste test trial prior to transplant. Right side shows the results of one taste 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

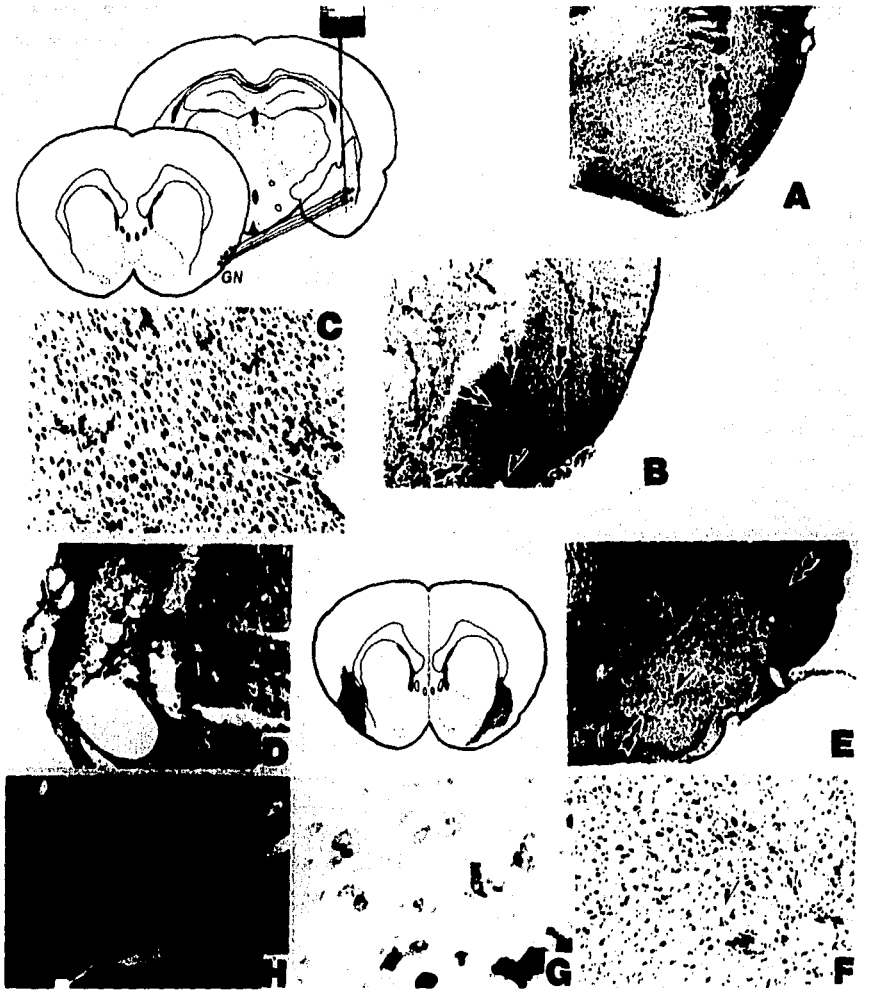

Fig. 2. Schematic representation of HRP injection in the amygdala and retrogradely labeled neurons within the gustatory neocortex (GN). A: a representative coronal section of amygdala HRP injection site. B: representative coronal sections of HRP-labeled control GN (arrows). D: the heterotopic GN grafts 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

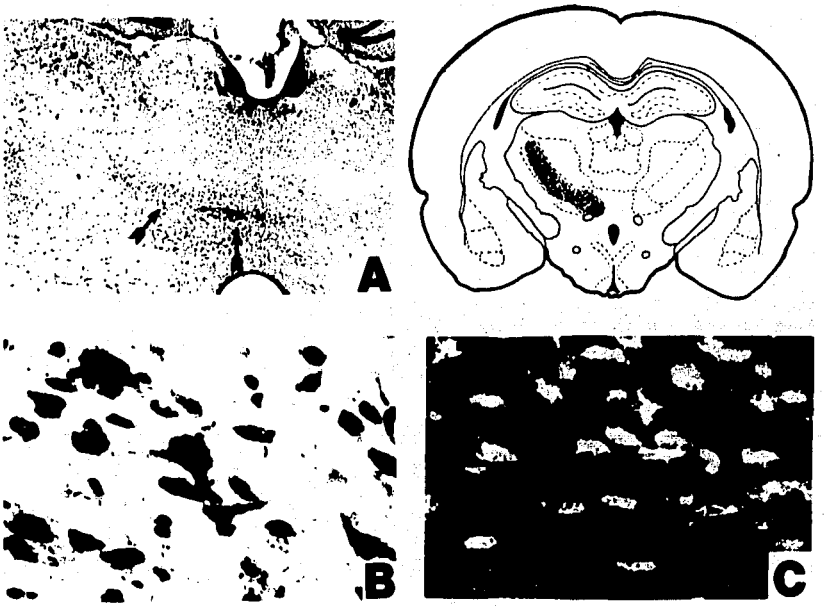

Fig. 3. A shows representative coronal section of HRP-labeled neurons (arrows) within ventromedial and ventroposteromedial (VM and VPM) nucleus of the thalamus. B is a magnification of A, showing HRP-labeled neurons. C is a dark-field illuminated magnification taken from the VM nucleus of a thalamus, 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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TIME DEPENDENCE RECOVERY OF TASTE AVERSION LEARNING BY FETAL BRAIN TRANSPLANTS IN GUSTATORY NEOCORTEX LESIONED RATS

Fernández-Ruiz, J.[1], Escobar M.L.[1], Piña A.L.[1], Díaz-Cintra S.[2], Cintra-McGleone F.L.[2] and Bermúdez-Rattoni F[1]*.

[1]Instituto de Fisiología Celular and [2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

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

Running Title: Time dependence recovery by fetal brain grafts.

* Please send all correspondence regarding this manuscript to:

Dr. Federico Bermúdez-Rattoni
Departamento de Neurociencias
Instituto de Fisiología Celular
Universidad Nacional Autónoma de México
Apdo. Postal 70-600
04510 México, D.F.
MEXICO

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 rats showing disrupted taste aversions due to gustatory neocortex lesions were employed. The lesioned animals received fetal cortical grafts, obtained from 17-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 30, 45 and 60 days post-grafts groups showed increased connections with the ventromedial nucleus of the thalamus and with the amygdala. In the group of 15 days post-graft there were absence of HRP labeled cells. In addition, the behavioral results were in agreement with an increased AchE time dependence reactivity and cell maturation detected by Golgi staining techniques. These results suggest that morphological maturity and reconnectivity between grafts and host tissue are needed for behavioral recovery in GN lesioned rats.

INTRODUCTION

The fetal brain transplant technique has been used recently as a very effective tool to ameliorate functional, and behavioral deficits, produced by either mechanical (16,28), chemical (15,17) or degenerative injuries to adult mammal brain (4,9,14).

Conditioned taste aversions (CTA) has been used widely as a model for study learning processes (11). 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 has been extensively studied (for review see, 11,18). 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 (18,20). Moreover, recently it has been described a reciprocal connections between the amygdala and gustatory neocortex (8,20).

Lesions of the gustatory neocortex region (GN) in adult rats leads to a behavioral impairment in both acquisition and retention of conditioned taste aversions (18,20). Recently, it has been demonstrated that cortical fetal brain transplants induce recovery of taste aversion learning in rats with gustatory neocortex lesions (1,8,29).

Nevertheless, the reasons how the brain transplants produce functional recovery, are not well understood. In this regard, several authors try to explain the behavioral improvement after fetal brain transplants in previous lesioned animals, by the

released of "trophic" factors (19). In contrast, other groups have pointed out that new connections between the graft and the host are responsible for the behavioral recuperation by the fetal brain transplants (6). Kesslak and coworkers (17) 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. According with these results, we recently demonstrated, with HRP technique, that the homotopic but not heterotopic brain transplants, were able to produced behavioral recovery and re-established connections with amygdala and with ventromedial nucleus of the thalamus, areas which connect with the gustatory neocortex (8).

Several authors have found connections between the cortical grafts and the host brain. That is, Floeter and Jones (10) reported that the cortical transplant projected some fibers to the thalamus, the contralateral cortex, the striatum and the hippocampus. Castro and coworkers (2) reported that different CNS regions, like; the basal forebrain, locus coeruleus and raphe, projected fibers to the cortical graft after ten months with intragraft injections of diamidino yellow and fast blue retrogradely transported fluorescent dyes (2). So it is clear that the cortical grafts are able to survive in the brain parenchyma, and receive and send projections to the host tissue (7,8).

With the aim to understand the processes underlying the behavioral improvement, we report in the present study the time

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

METHOD

Subjects

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

Surgery Procedure.

Large bilateral electrolytic lesions were made under pentobarbital anesthesia (50 mg/kg) to encompass the gustatory neocortex (AP=+ 1.2; L+5.3; V-5) in 30 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. 24 animals were used as unoperated controls.

Behavioral Procedure.

Following postoperative recovery (7 days) the experimental and control animals were deprived for 24 hr. 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 for 4 days.

The water consumption volume was taken everyday through a 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 the fifth day (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 baselines measures, in between (see 1). It has been demonstrated that rats can not discriminate between the NaCl and the LiCl flavor (23).

Transplant Procedure.

After the behavioral tests, the experimental animals were divided randomly in 4 groups (see below), and received homotopic cortical fetal brain transplants. Sixteen-day old fetuses were removed from the abdominal cavity of pregnant rats under anesthesia. The fetal brains were taken, and the temporo-parietal area (above the rhinal sulcus) were dissected under a microscope. The tissue were about 2 mm^3, then stereotaxically placed through a Hamilton microsyringe (100 ul), into the GN area with the same stereotaxic coordinates used to make the previous lesion. The experimental animals were randomly assigned to be behavioral retrained in fifteen (G15;n=8), thirty (G30;n=8), forty five (G45;n=6) or sixty (G60;n=8) days postgraft, (with the same procedure described above) with their respective temporal control groups (C15; n=6, C30; n=6, C45; n=6, C60; n=6).

Histological Procedure

At the end of the experiment, HRP histochemistry, Acetylcholinesterase histochemistry (AchE) and Golgi impregnation were made each in at least two rats per group (see below).

HRP Histochemistry. Horseradish paroxidase (Sigma VI) was dissolved in fast-green solution 2% (0.4 mg/10 ul). 16 Gustatory neocortex grafted subjects, from the four experimental groups (4 each) received the unilateral injection (0.5 ul) of the solution in the amygdala (n=2) and in the thalamus (n=2) ipsilateral to the graft. In addition, five control animals from C60 received a unilateral injection (0.5 ul) of the same solution, two in the amygdala and three 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 hr. survival period, rats were perfused intracardially 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 (21) 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.

Golgi Stain. 6 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 from rat's skull 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 period the solution was poured off and the tissue drained briefly on absorbent paper and transferred at 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 lied for 24 hours in absolute ethanol and ether (V/V). Following that, the tissue was advanced in a gradually more concentrated solutions of nitrocellulose (from 2% to 30%) in a total of 5 days at the maximum. The blocks were embedded in low viscosity 30% nitrocellulose and overnight hardened in a container with chloroform vapors.

The sections were seriately cut at 120 microns thickness on the sliding microtome, after that the sections were dehydrated in ethanol alcohol (70%, 80% and 95%), 10 min each. Then transferred to 98% isopropanol and terpineol (10 min. each). The sections were transferred to reagent grade xylene as soon as they became translucent. Finally the sections were mounted with synthetic resin.

Acetylcholinesterase histochemistry.

The rest of the subjects (2 of each experimental group) were anesthetized with pentobarbital and then perfused transcardially with the same formula described above for HRP perfusion. The brains were cut in slices 40 um thickness mounted and then immerse in the incubating solution as described by Paxinos & Watson (25) In the following day the slices were revealed in sodium sulfide pH 5 and mounted with synthetic resin and coverslided (25).

RESULTS

Behavior.

Simple ANOVA was done on the test day consumption volume for all groups, with post hoc group comparisons were appropriate using Student-Newmann-Keuls'tests (Fig.1). During the pregraft test trial, there were significant differences among groups ($F_{7,51}=11.4$ $P < .001$). As expected, all 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 ($P < 0.05$). In contrast, the G30, G45 groups, although consumed more saline solution than their

respective control, there were not significant differences among them. The G60 group showed a water intake suppression in the presence of the CS, and there was similar to its own control.

In addition, paired t test 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, G15 group 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 treated with HRP in the G15 experimental animals showed that there were not labeled HRP-cells in the grafted tissue, in the 4 experimental animals that received HRP injections in the thalamus or amygdala. In the graft tissue of G30 animals there were found scarce labeled HRP neurons. In contrast, in the 45 and the 60-days graft tissue, there were found a great number of labeled neurons, though not as many as in control tissue, as we have described previously (Table 1; Fig. 2) (8). In all the grafts in which there were found HRP labeled cells, the cell distribution inside the transplants did not follow any distinguishable particular pattern. We also found that the distribution of the labeled cells were independent from the injection site.

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 a 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 more neuronal density in the transplanted tissue particularly in those of 60 days. In general, the fetal transplants were adhere 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: 15 days; transplanted tissue showed scarce development of neurons and blood vessels. A 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. 30 days: Graft tissue appeared to be in a more advanced stage of development (see Fig. 3b). That is, neurons showed a great number of dendritic processes, growing in all directions from the cell body given a more defined neuronal

structure. The axons were apparent in the majority of the neurons. Blood vessels were found in the border and inside the transplanted tissue. There were found inside the transplants, many pyramidal and multipolar neurons. Glial cells were found in many parts of the transplanted tissue, without any regular pattern. 60 days: The transplanted tissue showed a great advance in the development of neurons and glial cells. (see Fig. 3c). Neurons presented a multipolar, piriform and triangular shaped-soma, 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 transplant tissue. The fibers were more abundant in the border of the transplant. There were found a lack of cortical lamination as can be compared with adjacent host tissue.

INSERT FIGURE 3 ABOUT HERE

Acetylcholinesterase Reactivity.

We found that the 15-days animals showed some labeled cells and there were few processes in the transplant. In the 30, 45 and 60 days groups there were an increased number of AchE fibers inside the transplants (see Fig. 4). These fibers formed patches along the grafts. The 15 days post-transplants groups did not show these AchE patches, since there were few AchE stained fibers (see 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 (see, Fig. 4).

INSERT FIGURE 4 ABOUT HERE

DISCUSSION

The behavioral data obtained in these experiments showed that the grafts taken 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 completed (G60; see Fig. 1), as the grafted group did not showed any significant differences with its own control (Fig. 1). The behavioral time dependence recovery was accompanied by time dependence histological changes. At 15-days post-graft the cortical transplants did not yet establish any demonstrable connections with thalamus nor with the amygdala through the use of HRP tracing technique (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 (29). In contrast, the 15 days posttransplant group showed an immature cell morphology with a few number of dendritic spines with Golgi staining technique (see Fig. 3).

Our results suggest that behavioral recovery should be accompanied by some maturation of the transplanted tissue. The maturity of the neurons could 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 (3). Moreover, other studies have also established that the thalamus of the adult brain could only establish few connections with the cortical transplants, from eight to twenty eight weeks after transplantation (12). 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 can be found in control animals (Table 1;B). 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 employment of the Golgi impregnation, in the 60 days grafts, we were able to see some fibers crossing the boundaries of the transplant to the host tissue, indicating a dynamic process of interaction between the transplant and the host tissue. Although, there were not a laminar arrangement in the grafts as can be seen

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 (22).

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 recent 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 malaise. Moreover, it was demonstrated with HRP histochemistry that the homotopic, but not the heterotopic, fetal brain transplants were able to re-established connections with the host tissue (8).

The demonstration of the AChE expression in the transplant in our results is supported by previous observations (13). Thus, other authors (24) 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 (24). In this paper, we are showing the time course of AchE graft expression. We observed a great reactivity of the 15-days soma in the graft, but with few processes. The number of all processes were increasing in the 30, 45 and 60-days post-graft, though the somas showed decreased AchE reaction with the time. By the moment, although we do not know the exact meaning of the AchE increased time reaction, some authors have proposed that the neurotransmitters enzymes have some axon guidance effects. That is, Robertson (26) 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 has been well established. Another, but not excluding, explanation is that there are ingrowth of axons from the basal forebrain or from the NBM nucleus to the cortical grafts as previously demonstrated by Ebner et al. (7).

Recently, several authors have demonstrated the presence of trophic factors delivered by specific systems. For example, Zhou and coworkers (30) 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 (31). 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 to explain the establishment of connections between GN and VPM, there is the possibility that this connections could be mediated by trophic factors (30,31). In our model, there are at least two potential sources of factors: the transplant by itself and the lesion-denervated host tissue (5). 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 coworkers (27). 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 dependence fashion. After 30 days the graft neurons started to establish connections with the thalamus or amygdala host tissues with HRP technique. 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 task in GN lesioned rats.

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

Table 1. Shows the cualitative 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.

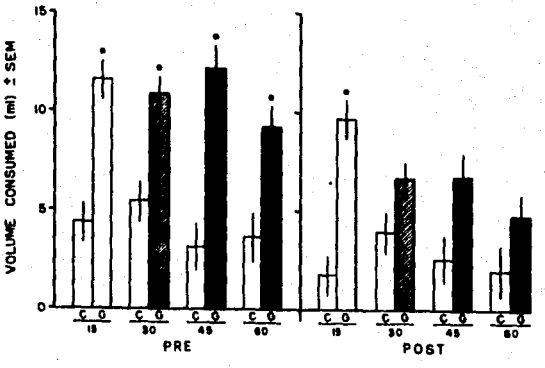
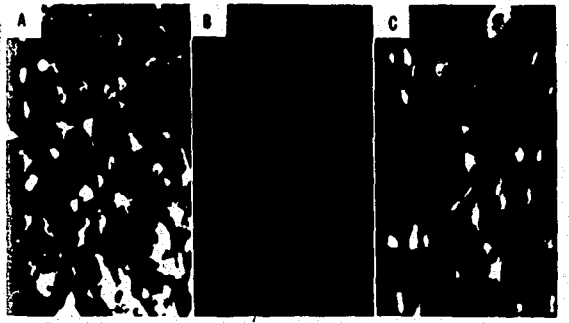
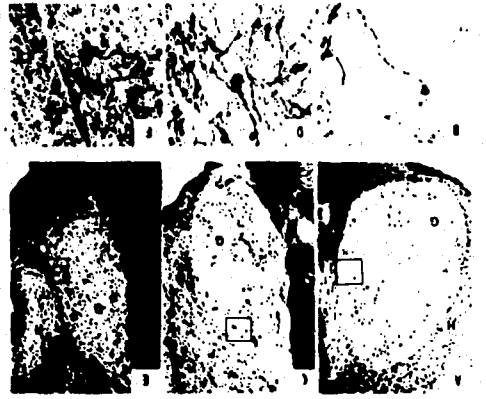
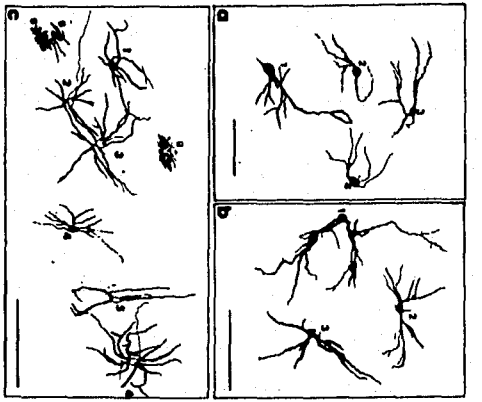


Figure 3. Camera lucida drawings of Golgi-rapid impregnated neurons from 15, 30 and 60 day old transplants. In a: Drawing of Golgi-rapid impregnated neurons from 15 day old transplant. Some neurons show a round shaped soma and dendrites supporting sparsely spines (see, cells 2,3). Cell 1 shows more spines 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.


A
B
C





DISCUSION GENERAL

Para comenzar la presente discusión, es conveniente hacer una breve recapitulación de lo que, considero, son los resultados más importantes que hemos obtenido en los artículos precedentes.

Primero confirmamos previos reportes (Kiefer, 1985; Lasiter y Glanzman, 1985; Bermúdez-Rattoni y cols. 1987) de la necesidad de la integridad de la corteza gustativa para obtener el CAS en ratas, ya que en dichos estudios se demostró que la lesión de la corteza gustativa era causa suficiente para provocar la pérdida del CAS.

Demostramos también que el transplante de corteza gustativa es capaz de sobrevivir e integrarse dentro del parénquima cerebral del hospedero (Bermúdez-Rattoni y cols. 1987; Escobar y cols. 1989; Fernández-Ruiz y cols. 1989), hallazgo que se ve confirmado por los resultados obtenidos en otros laboratorios no sólo con transplantes de corteza, sino inclusive en otros modelos (Mufson y cols. 1987; Fonseca y cols. 1988; Juliano y cols. 1989; Stenevi y y cols., 1985).

Con respecto a las conexiones del transplante con el hospedero, encontramos que el transplante homotópico estableció contactos con regiones con las cuales normalmente se relaciona, como son los núcleos talámicos VM y VPM, así como con la amígdala (Escobar y cols. 1989; Fernández-Ruiz y cols.). También observamos que los transplantes heterotópicos de tectum, degeneraron y no establecieron conexiones con el hospedero (Escobar y cols. 1989).

Finalmente encontramos que los transplantes son capaces de restaurar el CAS en una forma dependiente del tiempo. En el último artículo publicamos la recuperación de la expresión de la conducta a diferentes tiempos, siendo esta recuperación acompañada de la maduración neuronal así como del establecimiento de conexiones tanto con el tálamo como con la amígdala (Fernández-Ruiz y cols. 1989).

Una vez que se ha demostrado que los transplantes son capaces de reestablecer funciones perdidas por el hospedero (Fernández-Ruiz y cols.1989; Paredes y cols.1989; López García y cols. 1989) la pregunta a responder es cómo funcionan los transplantes para inducir dicha recuperación?.

TRANSPLANTES Y RECUPERACION EN EL HOSPEDERO: POSIBLES HIPOTESIS

1.- Factores neurotróficos.

Se han postulado diversas hipótesis a este respecto. Labbe y cols. basados en sus experimentos de lesión y transplantes de corteza frontal, en donde encuentran recuperación en periodos cortos de tiempo postransplante, proponen que "los transplantes pueden actuar liberando neurotransmisores, péptidos o factores que pudieran activar vías silentes o latentes que ya existieran en el sistema nervioso central de los organismos adultos" (Labbe y cols. 1983; Stein y cols. 1988). Para afirmar lo anterior, Labbe y cols. se apoyan también en los trabajos de Kesslak y cols. (1986a; 1986b) en los que encuentran que, los transplantes de corteza

frontal tanto de embriones como de adultos inducen una recuperación en una tarea de alternancia reforzada; y lo que es mas sorprendente, los transplantes de astrocitos pueden promover la recuperación conductual en el modelo de lesión en la corteza frontal sin necesidad de que se transplanten neuronas (Kesslak y cols. 1986).

Cabe mencionar en la presente discusión las investigaciones sobre el transplante de células cromafines de la médula adrenal que se han realizado para aliviar algunos síntomas en ratas con lesión de la vía nigro-estriatal. En dichos experimentos se ha encontrado que las células cromafines no establecen sinapsis, y ni siquiera extienden procesos en el hospedero, y aun asi inducen mejoría de algunos síntomas causados por la pérdida de conexiones entre la sustancia nigra y el cuerpo estriado. Se ha postulado que dicha recuperación se debe a la liberación continual de catecolaminas en el hospedero, es decir que en este caso el transplante funcionaría como una especie de bomba que libera la sustancia requerida por el organismo (Freed y cols. 1981).

Existe otra hipótesis que apoya el fenómeno de recuperación inducida por los transplantes. Esta hipótesis explica la recuperación sin necesidad de que se establezcan conexiones transplante-hospedero. Esta teoría se basa en el hallazgo del factor de crecimiento neuronal (FCN) (Levi-Montalcini 1987). Se ha demostrado que este factor es necesario para la supervivencia de algunos tipos celulares (Barde 1989), inclusive dentro del SNC (Hatanaka y cols. 1988).

En 1986 Sofroniew y cols. comunicaron que transplantes corticales evitan la atrofia de las neuronas colinérgicas del núcleo basalis magnocelularis (NBM) debida a daño cortical. Ellos postulan que el transplante de corteza provee de una influencia trófica al NBM, y que este factor previene su degeneración, siendo la naturaleza de este factor, posiblemente el mismo FCN (Sofroniew y cols. 1986). Los experimentos anteriores se vieron fortalecidos por el hallazgo casi simultaneo de Williams y cols. (1986) y Kromer (1987), que demostraron que la simple infusión de FCN es capaz de prevenir la muerte neuronal en el septum medial y en el núcleo de la banda diagonal de Broca, después de haber sido seccionado el fórnix.

La hipótesis del funcionamiento de los transplantes de tejido nervioso mediante la liberación de factores se ha ampliado a otros modelos de trabajo, como es la relación corteza-tálamo (Sharp y Gonzalez, 1986; Haun y Cunningham, 1984; Cunningham y cols.1987). Basados en el hecho de que tanto la remoción de la corteza occipital como la enucleación resultan en una degeneración casi total del núcleo geniculado lateral (NGL) (Cunningham y cols. 1979; Robertson y cols. 1989), Haun y cols. (1984) encontraron que transplantes de corteza occipital revertieron hasta cierto punto la disminución del NGL debida a la previa lesión de la corteza occipital. Más tarde, el mismo grupo (Cunningham y cols. 1987) reportó que el medio de cultivo de células de corteza occipital concentrado, embebido en geles e implantado en una avidad de la corteza occipital lesionada, tuvo el mismo efecto

sobre el NGL que el transplante de corteza occipital, y lo que es más, la adición de enzimas proteolíticas al medio de cultivo antes de la implantación del gel eliminó toda la actividad neurotrófica.

Esta hipótesis del funcionamiento de los transplantes se puede resumir diciendo que en estos modelos los transplantes pueden liberar factores neurotróficos que evitan la muerte neuronal en el hospedero; afirmación que se puede ligar a otros modelos de trabajo como es el caso de algunas enfermedades neurodegenerativas (Appel 1981) y sus posibles terapias, como la infusión de factores tróficos en sujetos viejos con deficiencias conductuales (Fisher y cols. 1987).

2.- Conexiones transplante-hospedero

No en todos los casos donde se ha encontrado recuperación conductual inducida por transplantes es posible sostener dicha hipótesis de trabajo; el mismo grupo de Cotman nos da la pauta para volver a mencionar la segunda hipótesis de trabajo acerca del funcionamiento de los transplantes. En 1988 Kesslak y cols. comunicaron que los transplantes de astrocitos purificados no indujeron recuperación conductual en animales previamente lesionados en el hipocampo; unicamente encontraron recuperación cuando transplantaron células hipocampales de feto, por lo que los autores postulan que en este caso es necesario el implante neuronal, a diferencia de sus hallazgos previos en corteza frontal (Kesslak y cols. 1986; Kesslak y cols. 1988).

Como ya se mencionó en la introducción, otros autores han

encontrado que es necesaria la innervación específica del hospedero por los transplantes para poder encontrar la recuperación conductual. Así, Dunnett y cols. (1982) encontraron recuperación conductual cuando el transplante de área septal innervó al hipocámpo hospedero, inclusive dicha recuperación se correlacionó con el grado de innervación colinèrgica del transplante al hospedero (Dunnett y cols. 1982).

Finalmente, los estudios que más apoyan esta hipótesis son aquéllos donde se encuentra la conectividad funcional entre el transplante y el hospedero, como es el caso de la integración de transplantes corticales en la corteza somato sensorial del sujeto receptor; por ejemplo mencionaré la integración transplante-hospedero observada por Bragin y cols., quienes comunican que los transplantes de corteza somatosensorial responden a la estimulación de las vibrisa (Bragin y cols. 1989); además de los estudios ya mencionados en la introducción (Ebner y cols. 1989; Sorensen y cols. 1989; Neafsey y cols. 1989).

Para algunos investigadores es difícil aceptar la formación de conexiones entre el transplante y el hospedero en un sujeto adulto, ya que desde tiempo atrás existe la idea de que el sistema nervioso central es, si no estático, sí muy poco plástico. Esta forma de pensar va cambiando, ya que recientemente se han encontrado fenómenos de plasticidad del SNC en diferentes niveles, que van desde el hallazgo del fenómeno de la potenciación a largo plazo (para revisión ver Collingridge y Bliss, 1987) -fenómeno que ha causado gran entusiasmo en la comunidad científica por ser un

buen modelo de plasticidad que se ha asociado al aprendizaje y memoria- hasta la observación del cambio de la representación sensorial en el homúnculo de la corteza somatosesorial del cerebro de macacos. En este modelo, Pons y cols. (1987,1988), investigaron la relación entre la corteza somato sensorial primaria (SI) y la corteza somato sensorial secundaria (SII) en macacos adultos. Ambas cortezas poseen un mapa somato sensorial del cuerpo del macaco, pero el procesamiento de la información no es igual; mientras que la corteza SI recibe la información directamente del tálamo, la corteza SII la recibe de la corteza SI. Estos autores demostraron que la ablación de una región del mapa somato sensorial de SI produce un "hueco" sensorial de esa misma región en SII. En dicho trabajo, se extirpó de SI la región somato sensorial que corresponde en el homúnculo a la región de la mano; al registrar en SII 24 horas después de dicha ablación, encontraron que la estimulación de la mano ya no resultó en una respuesta en SII, ya que se interrumpió el circuito tálamo-SI-SII. Sin embargo, cuando registraron en SII siete semanas después de la cirugía, encontraron que se reorganizó el mapa somatosensorial. La región en SII que antes de la ablación respondía a la estimulación de la mano, siete semanas después respondió a la estimulación del pie ipsilateral. No se detectó expansión de ninguna otra área, como podía ser por ejemplo el brazo. Estos experimentos demostraron elegantemente la gran capacidad de plasticidad que se puede encontrar en la corteza cerebral, especialmente la formación de conexiones que involucran un cambio en el mapa cortical (Pons y

cols. 1987, 1988).

En el modelo de recuperación del CAS por medio de transplantes de corteza gustativa, pueden aplicarse las hipótesis de funcionamiento de los transplantes ya mencionadas. Nosotros no encontramos recuperación conductual en periodos cortos de tiempo, pero sí encontramos conectividad con el hospedero y una buena integración del transplante; aunque esta afirmación parezca apoyar la hipótesis de reconectividad, es necesario mencionar un concepto acerca de los factores neurotróficos que se está gestando en algunos laboratorios. Cuando se observa el cerebro de una rata adulta, se observan núcleos y conexiones bien establecidas, sin embargo esto es en realidad un proceso dinámico. Este estado dinámico puede estar mediado por factores tróficos, los cuales pueden tener varias modalidades de funcionamiento, como por ejemplo, estimular la producción de procesos o la supervivencia celular o bien constituir señales para detener el crecimiento de las neuritas (Patterson, P.H. 1988). Como es que se establecen conexiones entre el transplante de corteza gustativa y el tálamo hospedero?. Una posibilidad es que el transplante de corteza gustativa envie axones a todos lados, hasta que encuentre un blanco que los acepte. Otra posibilidad es que el blanco denervado produzca factores que atraigan a sí innervación especifica, teoría que parece estar apoyada por algunos diseños experimentales (Cotman y cols. 1988; Lund y cols. 1988). La razón de porqué una zona denervada recibe inervación, o qué es lo que incita o estimula a una neurona en el adulto a innervar otra

zona del SNC no se sabe aún, pero empiezan a desarrollarse algunos modelos para contestar dichas preguntas (Zhou y cols. 1988).

Una vez implantado el tejido, las neuronas transplantadas pueden liberar factores o establecer conexiones con el hospedero. Vale la pena mencionar que en el hipocampo lesionado, se han implantado células secretoras de factor de crecimiento neuronal, evitando así la muerte de las neuronas que establecieron nuevas conexiones (Sofroniew y cols. 1986; Sharp y Gonzalez, 1986; Cunningham y cols. 1987) o estuvieron en contacto con el factor liberado, por ejemplo las células del área septal que son FCN-dependientes. Inclusive se ha postulado que los mismos neurotransmisores son capaces de funcionar como factores tróficos sobre las células blanco (Lipton y Kater, 1989).

Finalmente, cabe la posibilidad de que los transplantes sean integrados dentro de los sistemas funcionales del hospedero como parte integral de los circuitos que procesan la información (Bragin y cols. 1989; Ebner y cols. 1989; Sorensen y cols. 1989). En el modelo de transplantes en la corteza gustativa aún falta mucho por hacer. Los datos que hemos obtenido en esta linea de investigación no nos permiten postular cual es el mecanismo por el cual los transplantes inducen la recuperación conductual. Sin embargo sí es posible delinear algunos aspectos sobre los cuales podemos seguir avanzando, por ejemplo precisar las conexiones de las células transplantadas con sus posibles blancos, y profundizar el papel de algunos neurotransmisores y la expresión del CAS (López-García y cols. 1990).

CONCLUSIONES

En base a los resultados obtenidos podemos afirmar:

+ Los transplantes de corteza gustativa sobreviven y se integran al hospedero formando conexiones con regiones que normalmente son inervadas por dicha estructura; los transplantes de tejido heterotópico obtendido de la lámina cuadrigémina degeneran cuando son implantados en la región de la corteza gustativa

+ Los transplantes inducen recuperación conductual del CAS a largo plazo, este tiempo debe de ser por lo menos mayor a las dos semanas después del transplante de corteza gustativa.

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