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Fetal Striatal Tissue Grafts Into Excitotoxin-Lesioned Striatum: Pharmacological and Behavioral Aspects

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NORMAN, A B , M GIORDANO AND P R SANBERG. *Fetal striatal tissue grafts into excitotoxin-lesioned striatum Pharmacological and behavioral aspects* PHARMACOL BIOCHEM BEHAV **34**(1) 139–147, 1989 —In an excitotoxin animal model of Huntington's disease (HD), fetal striatal tissue transplants survive and grow in the host brain and reverse the behavioral, and, hence, functional deficits produced by the lesion In the present study we found recovery of apomorphine-induced rotation behavior in unilateral excitotoxin-lesioned rats indicating that the transplant reverses this functional pharmacologic deficit induced by the lesion It might, therefore, by expected that the transplanted fetal striatal tissue would possess similar pharmacological characteristics as the host striatum However, autoradiographic localization of D_1 and D_2 dopamine receptors demonstrated that the transplanted tissue expressed relatively small numbers of these receptor subtypes Furthermore, there was a relative deficit of [^{3}H]forskolin binding to the stimulatory guanine nucleotide regulatory subunit/adenylate cyclase complex in the fetal striatal tissue transplants Therefore, transplanted tissue which is neurochemically dissimilar to the host striatum is capable of reversing deficits in both drug-induced and spontaneous locomotor activity

Fetal striatal tissue grafts Striatum Excitotoxin lesions Dopamine receptors Forskolin Adenylate cyclase Rotation behavior

BRAIN tissue transplants may prove to be a useful approach to the treatment of a number of neuropsychiatric disorders in humans that are thought to involve selective degeneration of specific neuronal pathways in the brain (3) For example, Parkinson's disease involves loss of nigrostriatal dopaminergic neurons innervating the striatum Injection of fetal nigral cells into the striatum of animals with lesions of the nigrostriatal dopamine pathway has been demonstrated to ameliorate behavioral deficits observed in this model of Parkinson's disease (1, 4, 38). The neurodegenerative pathology of Parkinson's disease and the animal models of this disorder involve a relatively homogeneous population of dopaminergic neurons. However, relatively little work has been done to determine how transplanted tissue may replace complex neurodegeneration involving multiple neuronal types.

The injection of excitotoxins such as kainic acid, ibotenic acid and quinolinic acid into rat striatum produces a loss of multiple neuronal types and a reduction in the indices of cholinergic, GABAergic amino acid and peptidergic neurotransmitter systems (5, 32, 36) similar to the neurochemical deficits observed in the neurodegenerative disorder Huntington's disease (HD) Furthermore, excitotoxin lesions of the striatum also produce psychomotor disabilities and motor impairments (11, 30, 31) Great interest has been generated by recent studies which demonstrated in rats with excitotoxin lesions of the striatum that transplanted rat fetal brain tissue survives, grows and reinnervates the destroyed brain areas in adult rat brain (9, 20, 22). Similarly, behavioral and, hence, functional recovery in these models has demonstrated that transplanted material can be functionally integrated into the host brain (3, 7, 8, 9, 20–22, 27, 34, 35)

Although fetal striatal tissue transplants can reverse the spontaneous locomotor deficits induced by excitotoxin lesions of the striatum, relatively little work has been done to determine which neurotransmitter systems within the transplanted material are responsible for the behavioral recovery observed. Dopamine plays an important role in the behavioral responses mediated by the striatum (6) and dopamine receptor agonists or d-amphetamine produce rotational locomotor behavior in rats with unilateral KA lesions of the striatum (37) This is due to the destruction of the dopamine effector systems in the lesioned striatum (dopaminergic nerve endings are largely intact following KA lesions), while having normal intact dopaminergic neurotransmission in the contralateral striatum This produces an imbalance in dopaminergic

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neurotransmission in the basal ganglia It might be expected that following replacement of the striatal interneurons by fetal striatal transplants there would be a reduction in the relative asymmetry of dopaminergic neurotransmission in the basal ganglia This would be expected to reduce the degree of turning behavior in response to dopaminergic agonists Thus, amelioration of rotation behavior may provide a simple, sensitive and quantitative method for assessing recovery of pharmacological function following fetal striatal tissue transplants

METHOD

Animals

Adult male Sprague-Dawley rats weighing 250–300 g were housed individually and were given free access to food and water Twelve-hour light/dark cycles were held constant, starting at 0800 and 2000, respectively The timed-pregnant female rats were housed individually under the same conditions until used

Surgery

Striatal excitotoxin lesion Rats which received unilateral stereotaxic injections of kainic acid (KA) into the striatum were anesthetized with sodium pentobarbital (50 mg/kg), and 5 nmol of KA in a volume of one μl phosphate-buffered saline (pH 7 4) was stereotaxically injected into the striatum The coordinates were AP = 1 5, ML = 2 4, DV = 5 6, according to Paxinos and Watson (29) The KA was injected via a 26-gauge needle over a five-minute period After injection the needle was left in place for a further 2 min to allow diffusion of the drug solution Control rats underwent similar operations, but received microinjections of the vehicle alone Five min before the anesthetic, animals received atropine sulfate (Sigma Chemical Co) at a dose of 0 4 mg/kg IP After surgery they received 3 ml of dextrose (5%) IP in order to enhance postsurgery recovery Postoperatively, all animals were housed separately and given free access to food mash (powdered food pellets with Similac added) and water, and their body weight recorded daily

Transplant Operation

The transplanted rats received unilateral implants of day 17 fetal rat striatum into the KA-lesioned striatum The staged pregnant female rat was anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and prepared for laparotomy The mother was kept anesthetized until all fetuses had been dissected (2)

The dissection began by cutting the uterus with microscissors and gently pulling out the embryo with forceps The umbilical cord was sectioned and the fetus was placed in lactated Ringer's solution The extraction of the tissue did not last more than 20 min The dissection was performed under an operating microscope using Dumont No 5 dissecting forceps The embryonic brain was removed by peeling the skin and cranial cartilage away from the brain surface, and ensuring the complete removal of all attached meninges (2) The cortex was removed and peeled laterally in order to expose the half moon-shaped striatal tissue This tissue was aspirated into a glass capillary and lowered into the host striatum

The fetal tissue was then lowered stereotaxically by means of a glass syringe (Hamilton syringe, 50 μl) fitted with the glass capillary tube needle into the host striatum Coordinates were AP = 1 5 mm, ML = 3 4 mm and DV = 6 0 mm The fetal tissue was injected 1 mm lateral to the lesion coordinates to allow the tissue to be placed within the reduced striatal parenchyma which may enhance behavioral recovery (16,17) Tissue was injected at a rate of one μl per minute, the needle was left in place for one additional min and then it was raised 0 8 mm The procedure was repeated until 4 μl per side were delivered (approximately 1–1 5 mm^3 of fetal striatal tissue) The final stereotaxic coordinates for the DV dimension were 2 8 mm The needle was left in place for five additional minutes to allow diffusion, prior to slow retraction of the needle (2)

Apomorphine-Induced Rotation Behavior

Between four and six weeks after unilateral KA lesion, rats were placed into a Digiscan Activity Monitor and were injected SC with 0 5–1 mg/kg apomorphine The number and topography of rotations were also visually assessed in the open-field environment Rats were tested at three- to five-day intervals on at least three occasions prior to transplantation in order to obtain an accurate baseline for rotational behavior (39)

We divided the rotational behavior into three distinct categories based on visual observation of the topography of locomotion 1) Pivotal rotations were defined as ambulation in a complete circle around one or both stationary hind limbs The head and torso were rotated in the direction of rotation 2) Tight rotations were defined as locomotion in a circle, but using all four limbs for locomotion The head and torso were also rotated in the direction of locomotion 3) Walking rotations were defined as locomotion using all four limbs showing exploratory behavior, sometimes in a straight line, but ending at the starting point after continuous bias towards locomotion in one direction The head and torso were only occasionally rotated in the direction of locomotion

Four weeks after the lesion, the animals were transplanted as described above In this study the transplants were given 1 mm lateral to the lesion coordinates to compensate for shrinkage of the striatal parenchyma caused by the KA lesion (16,17) Control rats from the same lesion group were not given transplants and were challenged with apomorphine and tested in a manner identical to the transplanted animals Animals were again tested for rotational behavior at approximately five and ten weeks posttransplant to determine the effects of the transplant Observers were blind to the identity of the animals

Apparatus

A 16-beam Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH) was used The Digiscan system consists of Plexiglas monitor cages (40 × 40 × 35 cm) surrounded by horizontal and vertical sensors nondetectable by the animal These sensors direct a total of 48 infrared photo beams through the monitor cage The monitor cages are connected to a Digiscan Activity Analyzer which works in connection with an IBM XT computer to interpret the photo-beam interruptions and reveal many different aspects of locomotion (33) In addition, the Digiscan Monitor was coupled to a Comrex Comscriber 1 Activity Plotter In this study, the variables investigated were 1) horizontal activity the total number of interruptions of the horizontal sensors, 2) total distance the distance travelled by the animal in cm, and 3) average distance per movement episode the average distance the animal moved in cm during a movement bout

Histology

Following completion of behavioral analysis, the rats were anesthetized with pentobarbital (50 mg/kg IP) The chest cavity was exposed The diaphragm and rib cage were cut away to expose the heart and then perfused intracardially with cold saline The rats were then decapitated, the brain removed and immediately mounted on a cryostat chuck using mounting fluid and set using finely

powdered frozen CO_2. The mounted brains were frozen at −70°C until use Brains were mounted in a cryostat at −20°C and 32 μm thick sections cut Alternate brain sections were thaw mounted on gelatinized microscope slides, and three sets of slides were normally obtained One set was stained with cresyl violet and the second and third sets were used for determination of D_1 and D_2 dopamine receptor autoradiography, respectively In a separate set of experiments, rats received unilateral injections of KA (10 nmol) and four weeks later received transplants of day 17–19 rat fetal striatal tissue into the lesioned striatum. These rats were not subjected to any behavioral tests and did not receive injections of apomorphine Ten weeks following transplantation the rats were perfused and 32 μm brain sections obtained as described previously The three sets of slide-mounted sections were used for cresyl violet staining, the measurement of D_1 dopamine receptors or the measurement of [^{3}H]forskolin binding sites.

Receptor Autoradiography

For receptor autoradiography, the slide-mounted sections were dried at room temperature or at 2°C overnight in a sealed container with desiccant The slides were immersed in buffered solution containing either [^{3}H]SCH23390 or [^{3}H]spiperone for the measurement of D_1 and D_2 dopamine receptors, respectively The buffer consisted of 50 mM tris (pH = 7 7, 25°C) containing 125 mM NaCl and 5 mM $MgCl_2$ Ketanserin (40 nM) was also added to prevent binding of [^{3}H]SCH23390 (19) and [^{3}H]spiperone (24) to 5-HT_2 receptors The concentration of [^{3}H]SCH23390 or [^{3}H]spiperone was 1 or 3 nM or 1 nM respectively. Some of the slides for each ligand were incubated with the radioligand and 1 μM (+)butaclamol for the determination of nonspecific binding Slides were incubated in the appropriate solution for one hour and then placed into ice-cold buffer without radiolabel Five minutes later, the slides were placed into a second tray of ice-cold buffer and after five min were rapidly dipped into distilled water and rapidly dried using an air blow dryer. [^{3}H]Forskolin binding was assayed in 50 mM Tris buffer with 5 mM $MgCl_2$ and 10 mM NaF (28) and 200 mM sucrose was added to maintain an approximately isotonic solution Nonspecific binding was defined using 1 μM forskolin The slides were washed for 2 × 1 min in ice cold assay buffer and were rapidly dipped into distilled water and dried using an air blow dryer When the sections were fully dried (approximately 2 hours), the slides were placed in an x-ray cassette and in a photographic dark room, the slides were juxtaposed to a sheet of LKB ultrofilm and the x-ray cassettes sealed The x-ray cassettes were stored at 2°C for approximately 10 to 16 days for the [^{3}H]SCH23390- and [^{3}H]forskolin-labeled sections and 25 to 35 days for the [^{3}H]spiperone-labeled sections.

RESULTS

Apomorphine-Induced Rotation Behavior

As shown in Table 1, rats displayed rotation behavior in response to apomorphine which was normally restricted to one area of the open-field arena An illustration of this rotation behavior can also be seen in Fig 1A, C and D The rotation behavior was characterized by tight pivotal rotations in which one or both hind limbs remained stationary In our studies, approximately 60% of lesioned animals displayed turning. Those that did not were not used in the study

Five and ten weeks posttransplant, apomorphine-induced rotational behavior was reassessed using the same dose as was used prior to the transplant As shown in Table 1, the topography of the rotations changed from pivotal rotations to tight rotations and walking in a circle with both hind limbs used for locomotion.

TABLE 1

EFFECT OF RAT FETAL STRIATAL TRANSPLANTS ON APOMORPHINE-INDUCED ROTATION BEHAVIOR IN RATS WITH UNILATERAL KAINIC ACID LESIONS OF STRIATUM

	Peak Number of Rotations/ 5 min ± S E M	Mean % Pivotal	Mean % Tight	Mean % Walking
Lesion Only (n = 7)				
4 weeks postlesion	25 ± 5	43	45	12
9 weeks postlesion	21 ± 4	26	45	29
14 weeks postlesion	24 ± 2	38	51	11
Lesion and Transplant (n = 10)				
Pretransplant	26 ± 3	67	24	9
5 weeks posttransplant	19* ± 3	4	25	71
10 weeks posttransplant	15† ± 3	2	24	74

Significantly different from pretransplant values, *$p<0.02$, †$p<0.01$, two-tailed test

All rats were stereotaxically administered KA unilaterally into striatum Four to six weeks later, rats were placed in Digiscan Activity Monitors, and left for 20–30 min to habituate and were then challenged with apomorphine (0 5–1 mg/kg SC) Rotation behavior was assessed by visual observation and the number and type of rotations were quantified continuously during 5-min periods The definitions of pivotal, tight and walking rotations are given in the Method section Rat fetal striatal tissue was stereotaxically injected into the lesioned striatum of some rats while other rats received vehicle only (Lesion-Only group) Five and ten weeks posttransplant or after injection of vehicle, rats were rechallenged with the same dose of apomorphine that was previously used and rotation behavior assessed The values of pivotal, tight and walking rotations represent the mean percent from the total number of rotations during the entire period of visual observation

There was also a marked reduction in both the total number and maximal rate of rotations Furthermore, as illustrated in Fig 1B, locomotor activity was progressively less restricted to a small area of the arena corresponding to the change in topography of locomotion from pivotal to walking rotations. Apomorphine-induced stereotypic behavior either in one location or sniffing along a fixed path in the arena was also observed with greater frequency following the transplant In contrast to the amelioration of rotation behavior in the transplanted rats, most rats which received unilateral KA lesions, but did not receive transplants were not observed to improve In addition, there was no significant change in the topography of rotational behavior demonstrated by the animals between the 4- and 14-week postlesion test period (Table 1), although there was a tendency for an increase in pivotal rotations in some animals (Fig 1C and D)

The changes in the topography of locomotion are further demonstrated by the Digiscan Activity data As shown in Table 2, there is an approximately 12- to 16-fold increase in the mean total distance of rat locomotor activity following the transplant Furthermore, the average distance traveled during each locomotor episode increased approximately 2 5- to 3 5-fold

Receptor Autoradiography

As shown in Fig 2A, in a rat which displayed an excellent amelioration of the apomorphine-induced rotation, the transplanted tissue survived and grew within the lesioned striatum The transplanted tissue contained both large stained cells which had the appearance under high magnification of neurons and a large

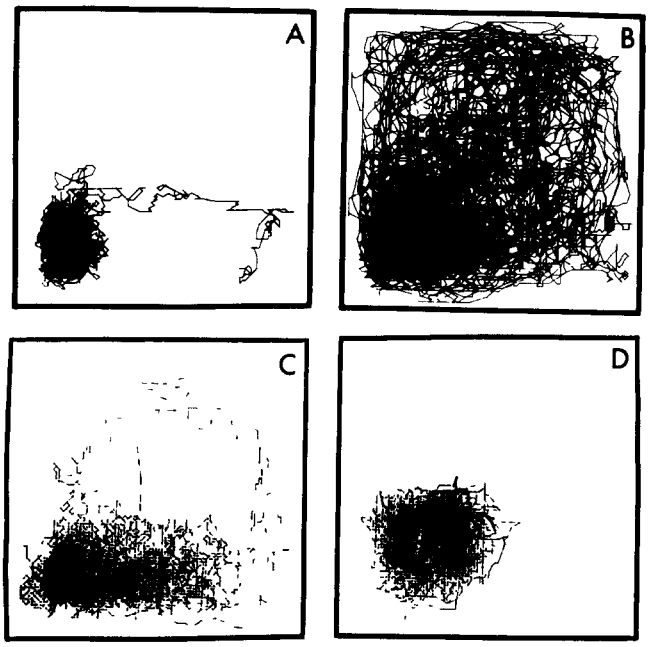

FIG 1 Activity plots of representative rats with a unilateral kainic acid lesion of the striatum (A and C) four weeks after the lesion and just prior to the transplant or sham transplant, (B) ten weeks after transplant of rat fetal striatal tissue and (D) 14 weeks postlesion The rats were placed in a Digiscan Activity Monitor and injected with apomorphine following a 20–30-min habituation period Activity was plotted for 45 min on a Comrex Comscriber I Activity Plotter The dimensions of the arena were 40 5 × 40 5 cm

number of small round densely stained cells which had the appearance of glia Thus, it appears reasonable to suggest that the transplanted striatal tissue itself is responsible for the amelioration of the apomorphine-induced rotation behavior The most parsimonious explanation of this phenomenon might be that the transplanted tissue possessed identical pharmacological characteristics as the original host tissue and merely replaced the function of the degenerated tissue However, the receptor binding data indicated that this does not appear to be the case. In adjacent brain sections to nissl-stained sections clearly showing a large transplant, the densities of both D_2 (Fig 2B) and D_1 (Fig. 2C) dopamine receptors within the transplant were markedly reduced compared to the normal host striatum. The marked reduction in the D_1 dopamine receptors relative to the normal striatum can also be seen in the transplant shown in Fig 3A This rat received a unilateral injection of 10 nmoles of KA which produced some damage of the cortex. This rat was not assessed for apomorphine-induced rotation behavior and did not receive any drug challenges Thus, the lack of D_1 dopamine receptors in the transplant is not dependent on exposure to dopamine receptor agonists Furthermore, there was a relative deficit of [^{3}H]forskolin binding to the stimulatory guanine nucleotide regulatory subunit/adenylate cyclase complex (28) in the fetal tissue 10 weeks posttransplant (Fig. 3B) As shown in the cresyl violet-stained sections (Fig 3C) adjacent to those used in Fig 3A and B, the transplant survived and grew within the host brain and contains cells which have the appearance of healthy neurons and glia There is no apparent glial scar surrounding the transplanted tissue Initial analysis of the autoradiograms using digital image analysis indicated that the density of binding sites for all three ligands within the core of the transplant was close to the observed levels of nonspecific binding

TABLE 2

EFFECT OF FETAL STRIATAL TISSUE TRANSPLANTS ON DIGISCAN MEASUREMENTS OF APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY IN RATS WITH UNILATERAL KAINIC ACID LESIONS OF THE STRIATUM

	Percentage of Pretransplant Levels		
Time Post-transplant	Horizontal Activity	Total Distance	Average Distance/Move
5 weeks	285 ± 72%	1285 ± 700%	364 ± 95%
10 weeks	251 ± 61%	1608 ± 1100%	429 ± 199%

Rats were individually placed in Digiscan Activity Monitors and left for 20–30 min to habituate Rats were then injected with apomorphine (0 5–0 75 mg/kg SC) and various activity variables were monitored for 5-min periods for 40 min Values shown represent the mean ± S E M percent of pretransplant levels (n = 5) The mean pretransplant values were total distance = 303 ± 254 cm and average distance per move = 5 3 ± 3 1 cm

DISCUSSION

Apomorphine-Induced Rotation

As well as the reversal of daytime hyperactivity and the more marked reversal of nocturnal hyperactivity observed following striatal transplants into excitotoxin-lesioned striatum, there have also been studies of the pharmacological effects of the transplants Dopaminergic neurotransmission is known to exert a tonic influence on striatal function and drugs which act on dopaminergic systems modulate locomotor activity in rats (6) Not surprisingly, the destruction of striatal interneurons, which are the target cells for nigral dopamine neurons, produces marked alterations in the response to d-amphetamine and rats with bilateral KA lesions of striatum demonstrate an increased hyperactivity in response to d-amphetamine (34) This increase in sensitivity to d-amphetamine is reversed following striatal transplants (34)

To further investigate the recovery of drug-induced behavioral functions, in the present studies we employed a different behavioral model using unilateral instead of bilateral excitotoxin lesions of the striatum [preliminary results have been previously reported (25,26)] In this model, rats will rotate in response to dopamine agonists either acting directly at receptors, i e , apomorphine, or indirectly by the release of dopamine from dopaminergic terminals, i.e , amphetamine or methamphetamine (37) The rotation behavior is presumably the result of the asymmetry in the dopamine effector cells, and a reduction in this asymmetry by replacing the lost circuitry with striatal transplants might be expected to ameliorate the rotation behavior in response to dopaminergic agonists This model provides a sensitive method for assessing recovery of pharmacological function

The data presented in this study clearly demonstrated that striatal transplants ameliorate the asymmetry in the dopaminergic effector systems Recent studies by Dunnett and co-workers (13,14) also using unilateral lesions demonstrated that there was recovery of pharmacological function eight months after the transplant The present study is in agreement with the results of Dunnett *et al* , but demonstrated that the recovery can be observed at a much earlier time point and is significant by five weeks following the transplant

The present study utilizes an open-field environment instead of a rotometer for measuring rotations The rotometer generally is used for measuring rotational behavior and consists of a bowl in which the rat is placed following injections with dopaminergic agonists This apparatus has the major advantage of being a very

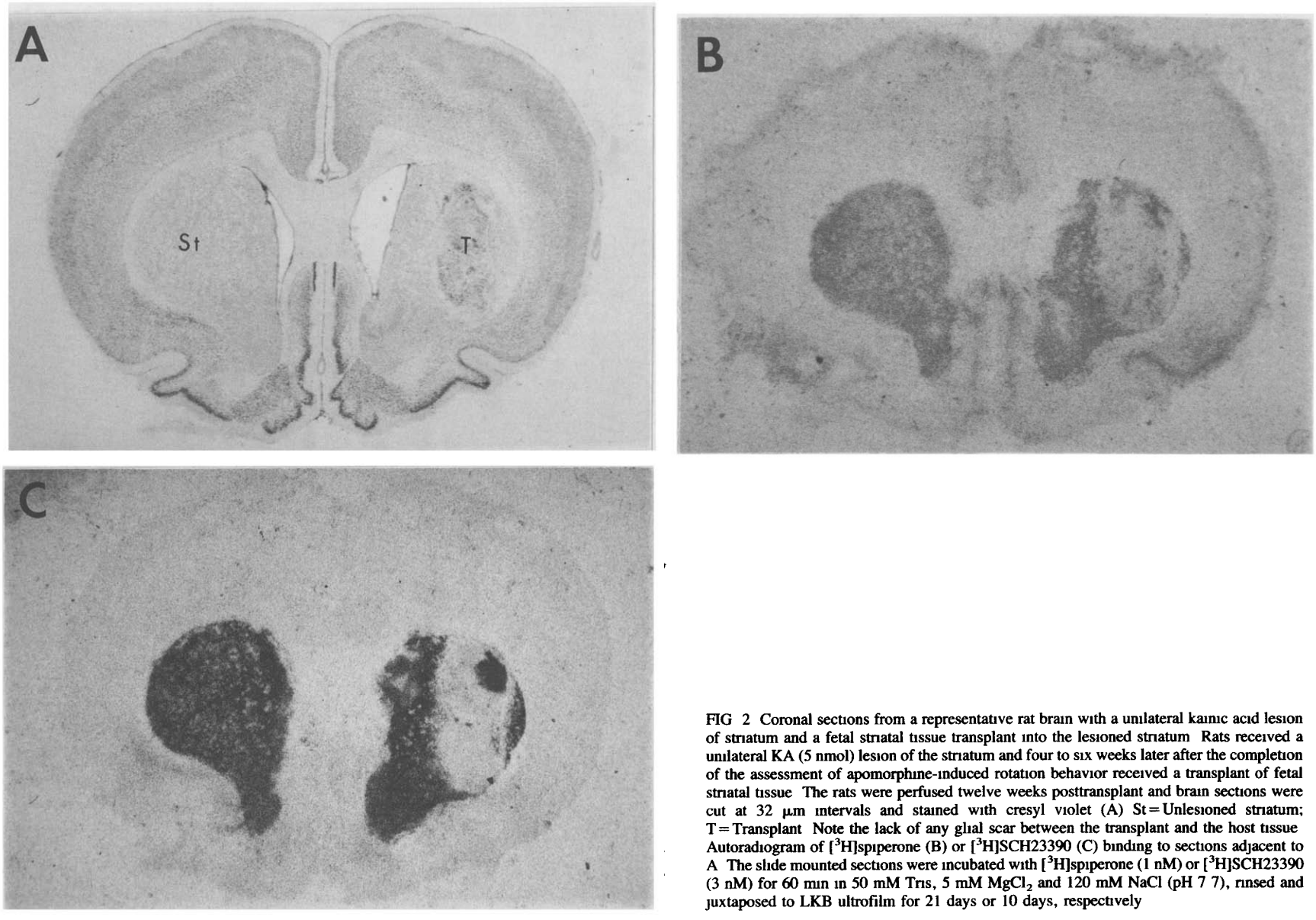

FIG 2 Coronal sections from a representative rat brain with a unilateral kainic acid lesion of striatum and a fetal striatal tissue transplant into the lesioned striatum Rats received a unilateral KA (5 nmol) lesion of the striatum and four to six weeks later after the completion of the assessment of apomorphine-induced rotation behavior received a transplant of fetal striatal tissue The rats were perfused twelve weeks posttransplant and brain sections were cut at 32 μm intervals and stained with cresyl violet (A) St = Unlesioned striatum; T = Transplant Note the lack of any glial scar between the transplant and the host tissue Autoradiogram of [^{3}H]spiperone (B) or [^{3}H]SCH23390 (C) binding to sections adjacent to A The slide mounted sections were incubated with [^{3}H]spiperone (1 nM) or [^{3}H]SCH23390 (3 nM) for 60 min in 50 mM Tris, 5 mM $MgCl_2$ and 120 mM NaCl (pH 7 7), rinsed and juxtaposed to LKB ultrofilm for 21 days or 10 days, respectively

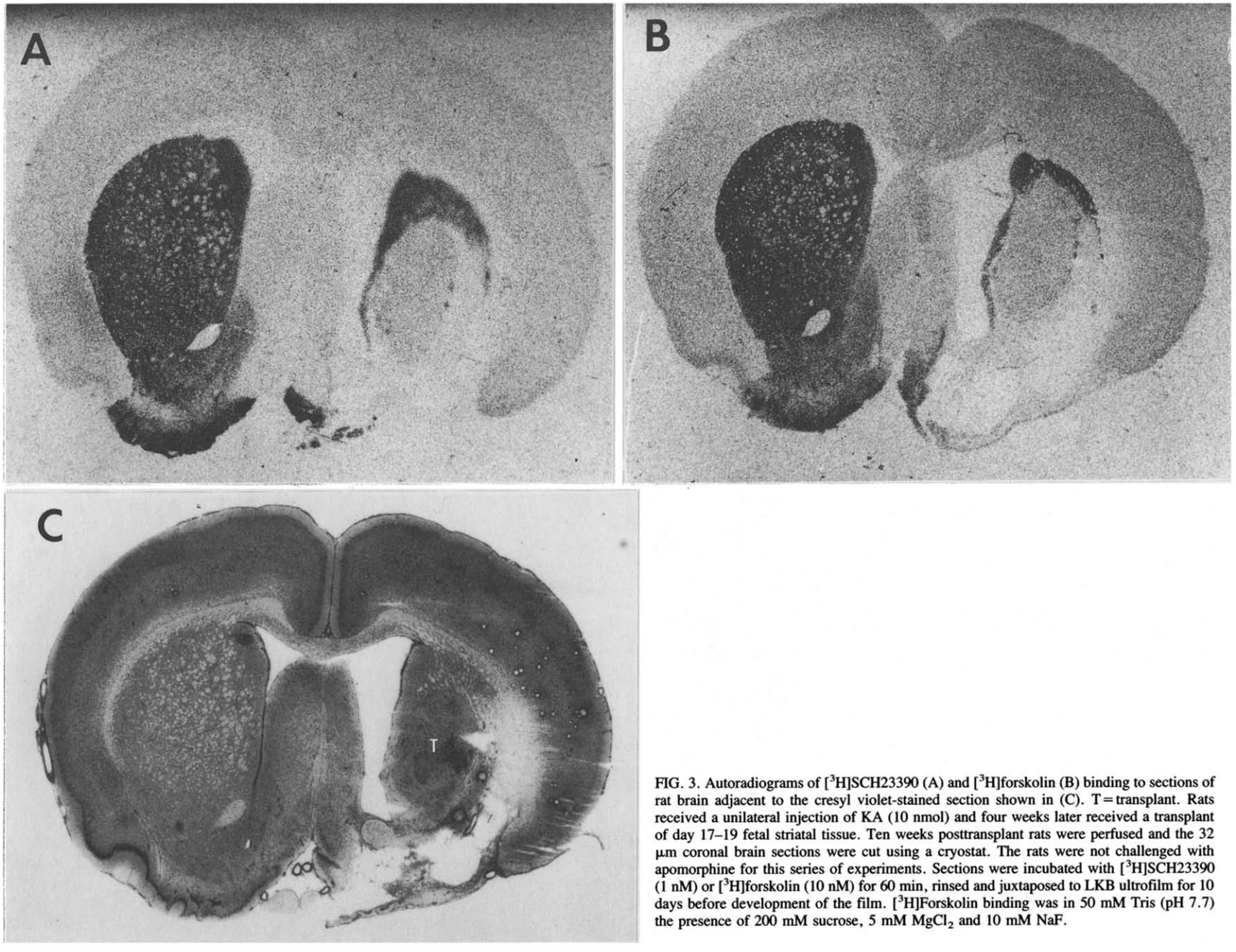

FIG. 3. Autoradiograms of [^{3}H]SCH23390 (A) and [^{3}H]forskolin (B) binding to sections of rat brain adjacent to the cresyl violet-stained section shown in (C). T = transplant. Rats received a unilateral injection of KA (10 nmol) and four weeks later received a transplant of day 17–19 fetal striatal tissue. Ten weeks posttransplant rats were perfused and the 32 μm coronal brain sections were cut using a cryostat. The rats were not challenged with apomorphine for this series of experiments. Sections were incubated with [^{3}H]SCH23390 (1 nM) or [^{3}H]forskolin (10 nM) for 60 min, rinsed and juxtaposed to LKB ultrofilm for 10 days before development of the film. [^{3}H]Forskolin binding was in 50 mM Tris (pH 7.7) the presence of 200 mM sucrose, 5 mM $MgCl_2$ and 10 mM NaF.

simple quantitative measure of rotational behavior and can be easily automated. However, it is important to note that most types of locomotion show up as rotation in this apparatus The present results clearly demonstrated that the rotation behavior was more complex in an open-field environment and the locomotor activity actually consists of different types of ambulation. These different types of ambulation cannot be observed in a rotometer and it is, therefore, important to be aware that useful information may not be observed if only a rotometer is used It can be seen in our data that the peak number of rotations decreased by approximately 30% and 50% five and ten weeks following the transplant, respectively However, there was an obvious transformation in the type of rotation, which changes almost exclusively from pivotal rotations to tight rotation and walking If a rotometer had been used, it is possible that only the relatively small change in the magnitude of rotation behavior would have been observed.

The intriguing possibility that distinct behaviors have discrete rates of recovery following the striatal tissue transplants is raised by the present data It appears that the topography of rotation changed rapidly and significant changes were observed within the first five weeks (26) Then, there was a decrease in the magnitude of the response at ten weeks with little further change in the topography It would be important to observe long term alterations in behavior following the striatal transplant similar to the study of Dunnett *et al.* (13,14).

We have previously (26) demonstrated that in rats which show marked amelioration of apomorphine-induced rotation behavior following the multiple striatal tissue injection procedure there was no evidence for a glial scar surrounding the transplant This has been observed in all the rats used in the present studies and can be seen in Figs 2A and 3C This indicated that there was no apparent barrier to neuroanatomical and functional integration between the transplant and host tissue. It is, therefore, interesting to speculate that the change in topography of locomotion might be due to relatively small amounts of integration on the border between the transplant and the host tissue, while reductions in the magnitude of response might require a later, more extensive integration of dopaminergic or other neurons from the host striatum with the striatal tissue transplants (3)

The amelioration of rotation behavior in the unilateral KA-lesioned and transplanted rats is similar to the results obtained using nigral transplants into unilateral dopamine deafferented striatum (12,15) However, in the latter studies, only a relatively homogeneous population of nigral dopamine neurons were transplanted into the striatum In the present studies, we were dealing with a more complex heterogeneous tissue consisting of a number of neuronal types and neurotransmitter systems These results suggest that the transplanted striatal tissue reduced the asymmetry in the dopamine effector systems in the rat striata

Receptor Autoradiography

The fetal striatal tissue transplants clearly reverse spontaneous behavioral deficits induced by excitotoxin lesions of the striatum [see reviews by Sanberg *et al.* (35) and Norman *et al* (27)]. Therefore, the striatal transplants are clearly functional We have now shown that these fetal striatal tissue transplants can also reverse the pharmacological deficits induced by the striatal excitotoxin lesions Thus, the behavioral asymmetry in dopaminergic systems is almost normalized by the transplants as can be seen by the amelioration of apomorphine-induced rotational behavior. If the transplanted striatal tissue produced a reversal of the pharmacological abnormalities produced by excitotoxin lesions of the striatum with respect to dopaminergic drugs, it might be expected that the transplanted tissue would also express dopamine receptors However, two studies exploring this hypothesis have produced diametrically opposite results Deckel *et al.* (8) reported a deficit of D_2 dopamine receptors in the fetal striatal transplant, which correlated with the lack of recovery of the hyperresponsiveness to d-amphetamine In contrast, Isacson *et al* (22,23) reported that although the D_2 dopamine receptors appeared to be patchy within the fetal striatal tissue transplants, the density of [^{3}H]spiperone binding within the patches was similar to the density observed in the normal striatum, and in a more recent study, Deckel *et al* (10) also reported patches of D_2 dopamine receptors within the transplanted striatal tissue Our present study indicated that while there was a marked recovery of apomorphine-induced behaviors, the transplant expressed few D_2 dopamine receptors Since it was also possible that apomorphine could be exerting its behavioral actions through the D_1 dopamine receptor subtype, we measured the density of D_1 dopamine receptors in the transplanted striatum. We have found that there are few, if any, D_1 dopamine receptors within the striatal tissue transplants. Furthermore, the deficit in [^{3}H]forskolin binding to the stimulatory guanine nucleotide regulatory subunit/adenylate cyclase complex (28) within the growing transplant further demonstrates that these transplanted neurons are neurochemically very dissimilar to the host striatal neurons

The factors which influence the expression of dopamine receptors within the transplanted tissue are unknown at present. However, the sex of the animal, the time between lesion and transplant, the time after transplantation, the excitotoxin used to create the lesion and the use of undissociated fetal striatal tissue as opposed to a disaggregated cell suspension are all variables between the studies of Deckel, Isacson and ourselves which might have a major influence on the expression of neurotransmitter receptors in the transplanted tissue The determination of the precise factors influencing expression of neurochemical and pharmacological characteristics of the transplanted tissue will require extensive investigation The studies of Isacson and co-workers who reported the presence of receptors used dissociated cell transplants, while those studies by ourselves and Deckel who report the relative absence of D_2 dopamine receptors within the transplant used whole undissociated tissue transplants which were not treated with enzymes, etc. However, it is important that irrespective of the neurochemical characteristics of the growing transplanted tissue, the transplant is able to reverse deficits in both drug-induced and spontaneous locomotor activities The mechanism by which these actions are elicited is not currently understood, but these results may have important implications for a therapeutic intervention in HD based on neural transplantation. That neurochemically dissimilar tissue is able to reverse the functional deficits may indicate that tissues other than fetal striatum may also reverse functional deficits

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