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Locomotor Hyperactivity: Effects of Multiple Striatal Transplants in an Animal Model of Huntington's Disease

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SANBERG, P. R., M. A. HENAULT AND A. W. DECKEL. *Locomotor hyperactivity: Effects of multiple striatal transplants in an animal model of Huntington's disease.* PHARMACOL BIOCHEM BEHAV 25(1) 297–300, 1986.— Huntington's disease is characterized by gross degeneration of the intrinsic neurons of the striatum, restless hyperkinetic choreiform movements and dementia. Rats which received injections of kainic acid have provided an extremely viable model for this extrapyramidal movement disorder. The present preliminary report investigated the effects of multiple homotopic transplantations of normal fetal Day 17 striatal ridge tissue into the lesioned striatum of male kainic acid-treated rats. Nine weeks after transplantation, the spontaneous nocturnal hyperkinetic locomotor abnormalites as measured by horizontal activity and total distance travelled were attenuated in the striatal transplanted animals compared to sciatic nerve transplanted controls. Similarly, the exacerbated response to d-amphetamine exhibited by the animal model was attenuated in the striatal transplanted animals. The striatal transplants reconstructed much of the gross morphology of the lesioned striatum, although acetylcholinesterase was found to be reduced.

Locomotor activity Huntington's disease Kainic acid Neural transplantation Striatum d-Amphetamine Digiscan

HUNTINGTON's disease (HD) is a hereditary condition which is characterized by a gross degeneration of the intrinsic neurons of the striatum. Restless hyperkinetic choreiform movements and dementia are the primary symptoms of the disease [16]. Because the pathophysiologic basis for the symptoms of HD are still not fully understood, animal models of the disorder have been developed in order to define the possible mechanism(s) involved. To this end, rats which received injections of kainic acid have provided what appears to be an extremely viable model of HD. The rats display many of the neuroanatomical, neurochemical and behavioral abnormalities seen in patients with HD [17]. Paramount among the behavioral deficits seen in these animals, is a pervasive hyperactivity which occurs primarily during the awake cycle or nocturnal period of the rat. Most patients with HD show greater abnormal movements during their awake period and when aroused [16,21]. While there are obvious differences between the choreiform movements of humans and the topography of the animal locomotion in kainic acid striatal lesioned rats, this model has proven useful as a screening method for investigating potential therapies for HD.

This study investigated a recent approach for the possible treatment of HD; namely the effects of homotopic transplantation of normal fetal striatal tissue into the diseased neostriatum of kainic acid lesioned rats. Kimura, McGeer and McGeer [10], McGeer, Kimura and McGeer [12] and Schmidt, Bjorklund and Stenevi [20] found that grafts of fetal or neonatal striatal tissue grew robustly in the striatum of rats previously injected with KA, and could reverse the neurochemical changes. Subsequently, it was found that fetal implants could also reverse some of the behavioral deficits caused by kainic acid [1] or ibotenic acid [7] induced lesions of striatal neurons. Deckel *et al.* [1] indicated that fetal transplants in female kainic acid lesioned rats can actually partially reverse some of the locomotor hyperactivity seen in this animal model of HD. Subsequently, they demonstrated that the amount of recovery of function was correlated with the number of transplanted neurons surviving in the striatum [3]. In that study only a small amount of fetal tissue was injected into one area of the striatum in female rats. Recent findings have demonstrated improved recovery of function in 6-OHDA lesioned rats after multiple injections of embryonic tissue into the denervated striatum [4,5], and the existence of topographical relationships for behavioral functions within the striatum [15]. Therefore, the present study observed the effects of multiple transplants in male rats, in order to determine if more complete recovery of function, than that previously found, could be obtained.

METHOD

Adult male rats received bilateral injections of kainic acid (2 nmole/0.8 μl saline) as previously described [3,18]. This neurotoxin lesioned the intrinsic neurons within the dorsal and ventral striatum while leaving intact the afferents and

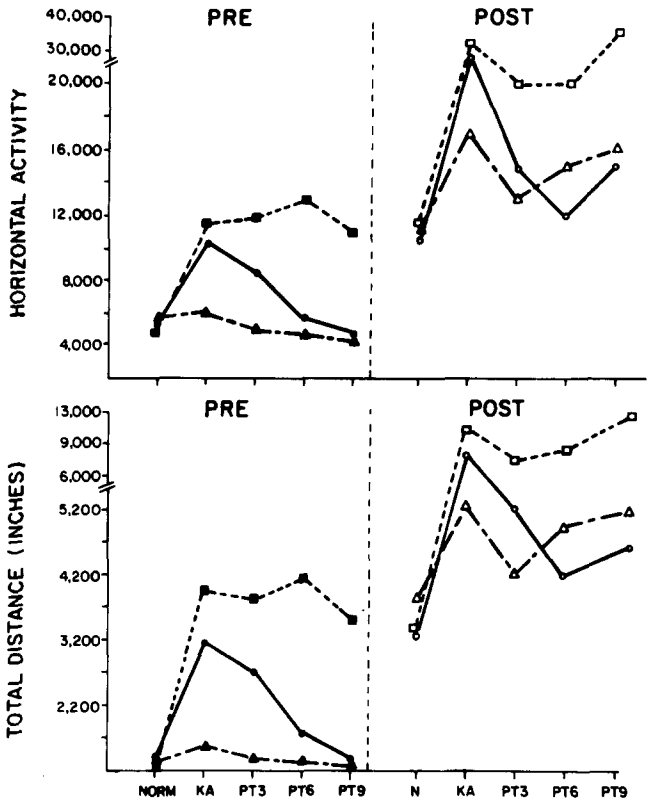

FIG. 1. Mean nocturnal horizontal activity and total distance travelled between rats which received sham lesions with sham transplants (triangles), kainic acid lesions with sham transplants (squares), or kainic acid lesions with striatal transplants (circles n=7). PRE represents the pre-amphetamine injection period (2200 to 2300 hr), and POST represents the post-amphetamine (1.0 mg/kg) injection period (2300 to 2400 hr). NORM represents the behavioral testing trial at one week prior to the kainic acid lesion. KA represents the behavioral testing at four weeks after the kainic acid lesion. PT3, PT6 and PT9 represents the behavioral testing at three, six and nine weeks post-transplantation, respectively. Statistical analysis revealed that both the kainic acid groups (squares and circles) were significantly hyperactive from the sham-sham group (triangles) at the post-lesion period (KA), whereas only the kainic acid-sham group (square) was hyperactive from the other two groups post-transplantation.

traversing pyramidal fibers. Four weeks post-lesion all rats (except sham-transplanted animals) received bilateral implantations of Day 17 fetal striatal ridge tissue as described by Deckel *et al.* [3]. For the multiple transplants, four 1 μl injections of homotopic tissue (approximately 1–1.5 mm^3) were injected, with each deposit separated 0.8 mm dorsal to the previous deposit. The first deposit was begun in the ventral striatum at the coordinate of DV = −4.7 mm according to the König and Klippel [11] atlas. Following each injection, the pipette was kept in position for one minute to allow the proper diffusion prior to the slow retraction of the needle. After the final injection, the pipette was kept in position for an additional five minutes. Sham-transplanted rats received bilateral implants of adult sciatic nerve [6]. Another sham-nontransplanted group consisted of essentially normal animals with sham operations.

At three, six, and nine weeks post-tranplantation the rats were individually tested in computerized Digiscan Animal Acitivity Monitors (Omnitech Electronics, Inc.) as described elsewhere [19]. The locomotor activity of the three groups of animals was assessed during the nighttime at peak activity periods (22:00–23:00 hr) according to preliminary studies. At 23:00 hours the rats were injected with either d-amphetamine sulfate (1.0 mg/kg) or saline, and locomotor behavior was monitored for one-hour. Food and water were available during the entire testing period. In this preliminary report only the horizontal activity (number of infrared beams broken by the animal) and the total distance travelled were analyzed by ANOVA.

At the conclusion of the experiment, rats were anesthetized with IP injection of Nembutal and then intracardially perfused with 0.9% physiological saline followed by 0.9% solution of buffered formalin. The brain was extracted, rinsed in demineralized water, and stored at 4 degrees C until it could be sectioned. Selected brain sections were then histologically evaluated by staining alternate coronal sections with cresyl violet or acetylcholinesterase techniques [14].

RESULTS

The results of the nocturnal ambulatory behaviors (horizontal activity and total distance) demonstrated that the kainic acid lesioned rats with sham transplants were consistently hyperactive during the nine week testing period compared to sham controls (Fig. 1). However, the hyperactivity exhibited by the striatal transplant group prior to transplantation decreased gradually following implants until they reached control levels nine weeks later. Furthermore, when tested with amphetamine (1.0 mg/kg) the exaggerated response exhibited by the sham transplant kainic acid lesioned group was not present in the striatal transplant group at nine weeks post-tranplantation. Very preliminary analyses of rearing, stereotypic and rotational behaviors also showed a similar pattern of results.

Upon histological examination the striatal transplants reconstructed much of the gross morphology of the lesioned striatum in recovered animals (Fig. 2). However, acetylcholinesterase was much less apparent within the transplanted tissue than the host brain. In fact, as can be seen in Fig. 2 the decreased amount of acetylcholinesterase staining offered a good way to illustrate the transplant from the host tissue.

DISCUSSION

These results support previous research indicating that transplanting homotopic fetal striatal tissue into the lesioned striatum of adult animals may be a useful technique for producing recovery of function in similar neurodegenerative human disorders, such as Huntington's Disease [1–3, 7–10, 12, 20]. Deckel *et al.* [1] first demonstrated that the daytime hyperactivity found during the habituation period in an open-field by female kainic acid lesioned rats could be significantly reduced by a single injection of Day 17 fetal striatal tissue. Earlier studies have not found any abnormalities in the general daytime habituation of KAL animals when placed in photocell cages [17,18], although a pervasive nocturnal hyperactivity has been repeatedly demonstrated [7, 9, 13, 17]. It is possible that the significant daytime effects reported by Deckel and his colleagues [1–3] are due to the superior sensitivity of the Digiscan activity monitors over conventional photocell cages [19], and/or sex differences in the locomotor response to the lesion, since previous studies have used male rats (e.g. [13, 17, 18]).

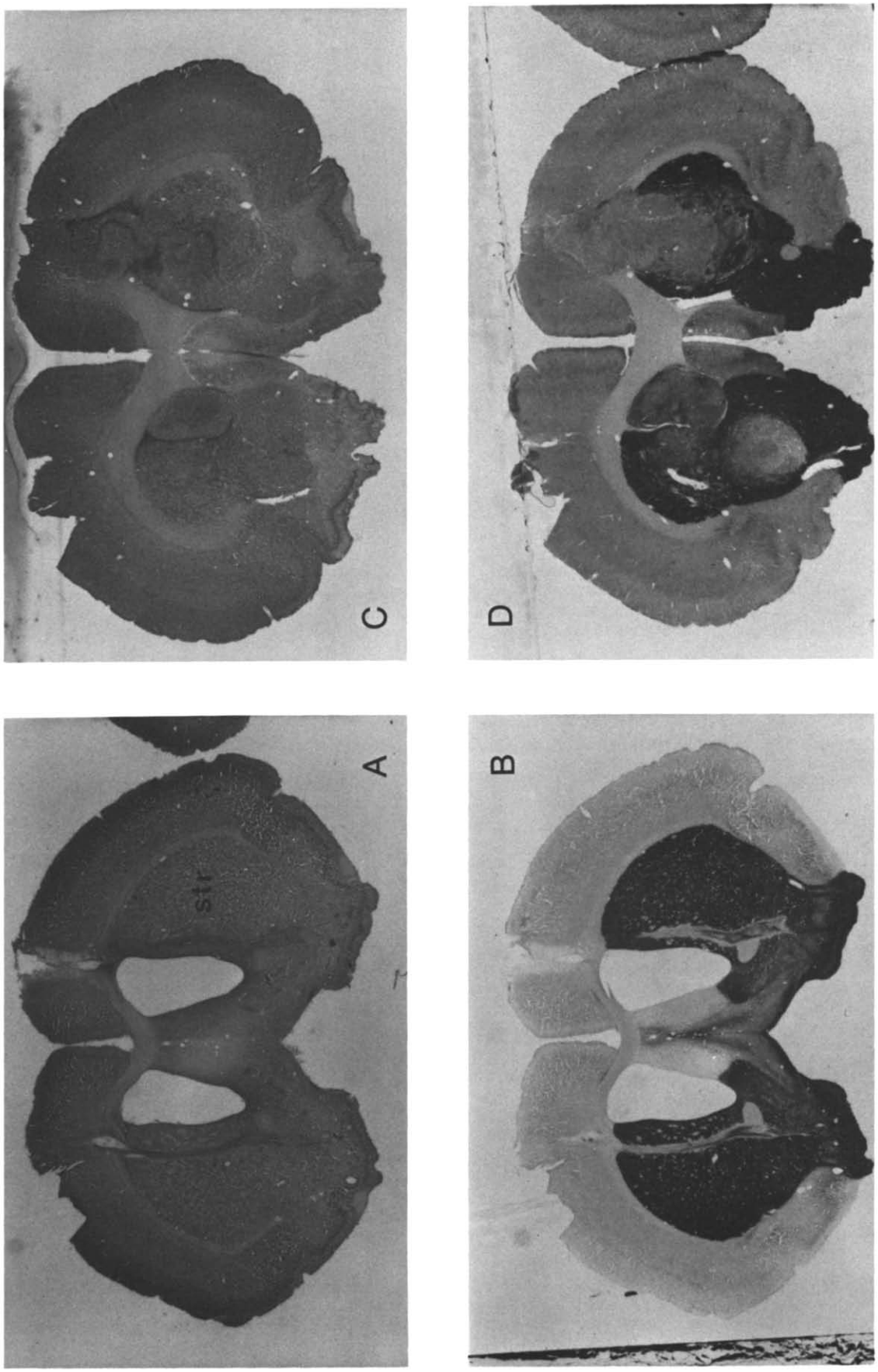

FIG. 2. Photomicrographs of coronal sections through the medial rostral-caudal extent of the striatum (str) in rats which received kainic-acid induced striatal lesions followed, four weeks later, by intrastriatal injections of sciatic nerve (i.e., sham group, A and B) or fetal striatal tissue (i.e., experimental group, C and D). Brain sections were cut at 50 microns and alternate sections were stained with cresyl violet (A and C) or acetylcholinesterase (B and D) techniques (see [14]). The sham brain (A and B) depicts an animal with one of the smallest kainic acid lesions. It is shown because it illustrates the bilateral injections of sciatic nerve tissue quite well. In this study using lot number 32F-0687 of kainic acid (Sigma) the lesions were typically almost twice as large as that shown in A and B. The experimental brain (C and D) depicts a typical animal with a large transplant that has integrated with the host striatum and filled up the dilated ventricles resulting from kainic acid. This animal shows some transplant tissue that encroached into the cortex on one side. In spite of this, the represented experimental animal demonstrated excellent recovery of function.

Isacson *et al.* [7] demonstrated that male rats with ibotenic acid-induced striatal lesions showed persistent nocturnal hyperactivity which could be significantly reduced with fetal striatal transplants. The present preliminary findings confirm these results and extend them by demonstrating that large fetal transplants produced by multiple injections can completely reverse some aspects of nocturnal locomotor hyperactivity in rats with large kainic acid-induced striatal lesions. It was also found that the characteristic hyperresponsiveness to the locomotor stimulating effects of d-amphetamine exhibited by the kainic acid-lesioned rats [17,18] could be attenuated by the large striatal transplants. This effect was not found in an earlier study by Deckel *et al.* [2] using female rats and smaller transplants. The difference between these two studies may again be due to sex differences and/or that large transplants are needed for recovery of dopamine-stimulated behaviors. In fact, Deckel *et al.* [3] demonstrated that the neuronal density of the transplant predicted the extent of behavioral recovery.

Using tritiated spiperone autoradiography, Deckel *et al.* [2] demonstrated that while fetal striatal transplants survived robustly, few dopamine receptors were found in them, suggesting that the reinstatement of the disturbed nigral-striatal circuitry is not normal following transplants. Similarly, the present results demonstrated that acetylcholinesterase is greatly reduced in the surviving transplants, suggesting that the reinstatement of cholinergic systems may not occur normally either. While these findings may suggest that the transplants are not integrating neuroanatomically within the host brain, other studies have found increased activity of the neurotransmitter synthesizing enzymes, choline acetyltransferase and glutamic acid decarboxylase, following striatal transplants [8,10]. This suggests that the transplants increased the pool of cholinergic interneurons, and GABAergic efferent neurons within the lesioned striatum. Furthermore, Isacson *et al.* [8] demonstrated that the striatal transplants form GABAergic efferent connections with the globus pallidus.

It is evident that fetal striatal transplants can produce remarkable recovery of function of both locomotor [1–3, 7, 9] and more complex psychological tasks [9] in an animal model of Huntington's disease. However, further biochemical and neuroanatomical studies are needed in order to clarify (1) what mechanisms underlie the behavioral recovery of function, (2) the parameters for successful transplants, and (3) the extent of synaptic recovery.

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