Feeding behavior in dopamine-deficient mice

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Mice that cannot make dopamine (DA), a condition caused by the selective inactivation of tyrosine hydroxylase in dopaminergic neurons, are born normal but gradually become hypoactive and hypophagic, and die at 3 weeks of age. We characterized the feeding and locomotor responses of these DA-deficient (DA−/−) mice to 3,4-dihyroxy-L-phenylalanine (L-DOPA) to investigate the relationship between brain DA levels and these complex behaviors. Daily administration of L-DOPA to DA−/− mice stimulated locomotor activity that lasted 6 to 9 hr; during that time the mice consumed most of their daily food and water. The minimal dose of L-DOPA that was sufficient to elicit normal feeding behavior in the DA−/− mice also restored their striatal DA to 9.1% of that in the wild-type (WT) mice at 3 hr; then DA content declined to <1% of WT levels by 24 hr. This dose of L-DOPA induced locomotor activity that exceeded that of treated WT mice by 5- to 7-fold, suggesting that DA−/− mice are supersensitive to DA. Unexpectedly, DA−/− mice manifested a second wave of activity 24 to 48 hr after L-DOPA treatment that was equivalent in magnitude to that of WT mice and independent of DA receptor activation. The DA−/− mice approached, sniffed, and chewed food during this second period of activity, but they ate <10% of that required for sustenance. Therefore, DA−/− mice can execute behaviors necessary to seek and ingest food, but they do not eat enough to survive.

Rats treated with 6-hydroxydopamine (6-OHDA) and monkeys treated with *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have been studied extensively as models of Parkinson's disease because both neurotoxins can be used to destroy the same nigrostriatal dopaminergic neurons that die during the progression of the human disease (1, 2). In animal models and in human Parkinson's disease, behavior is generally unaffected until >80% of the dopamine (DA) is depleted. Hypoactivity, deficits in motor coordination, and failure to eat or drink become evident with more severe DA depletion (3–5). The aphagia and adipsia of rats treated with 6-OHDA can be life-threatening unless they are kept alive by intragastric feeding. With this treatment, the rats gradually become more active, and eventually they eat enough food without assistance to stay alive and gain weight. Compensation for the feeding deficit in lesioned animals is thought to occur through improved efficacy of dopaminergic signaling, because DA levels remain low and a subsequent blockade of tyrosine hydroxylase activity will reinstate aphagia and akinesia (6). Although feeding returns to normal, deficits in feeding responses can be elicited by physiological challenges (4, 5). Neonatal rats with 6-OHDA lesions continue to suckle and can be weaned onto normal chow without major impairments of feeding, despite nearly complete elimination of DA. This suggests that compensatory mechanisms are even more efficacious in young rats (2, 7).

Parkinson's disease differs from the rat model in that the symptoms generally become more severe with age, and, as neurons continue to die, tremor is a prominent feature, but aphagia is generally not a major symptom. Food intake may decline as the disease progresses, but it is not clear whether that is attributable to decreased motivation to eat, difficulty in maneuvering eating utensils, or nonspecific depression. The feeding deficit in the rat model has been attributed to sensorimotor impairment and/or a loss of motivation to eat; however, the mechanisms have not been elucidated. Delivery of DA agonists and antagonists to different sites in the brain has identified specific regions that influence feeding behaviors (8, 9), but they have not indicated where the DA action is essential. Furthermore, the genetic approach of inactivating individual genes encoding DA receptors or transporters has not revealed any striking effects on feeding behavior (10–13). Thus, the specific roles of DA in feeding remain enigmatic.

Gene-targeting techniques were used to inactivate the tyrosine hydroxylase gene in dopaminergic neurons, sparing the production of DA as a precursor for norepinephrine and epinephrine (14). Initial studies in these DA−/− mice revealed that DA is important for control of locomotion, muscle coordination, feeding, and probably other behaviors. The variable extent of 6-OHDA lesions, along with a gradual and variable duration of compensation, makes the study of some behaviors difficult in that model. In contrast, the specificity of genetic lesion (resulting in reproducible and stable behavioral phenotypes) provides favorable background for ascertaining where in the brain, and, ultimately how, DA modulates various behaviors. Prolonged restoration of feeding to physiological levels by pharmacological or gene therapy approaches is rarely described in the 6-OHDA-lesion model, although 3,4-dihyroxy-L-phenylalanine (L-DOPA) and DA agonists can reverse some of the symptoms transiently (15–17). Notable exceptions are studies in which transplantation of dopaminergic tissue revealed that local production of DA in striatum could prevent aphagia induced by 6-OHDA (18, 19). Routine pharmacological treatment induces feeding in DA−/− mice, and the feasibility of long-term rescue of feeding behavior (over a year) by gene therapy has been established (20). Our goal is to map more precisely where DA is required to restore various behaviors, including feeding. A prerequisite to such studies, however, is characterization of behavioral deficits of DA−/− mice.

Materials and Methods

Mice. DA−/− mice were created as described (14). The wild-type (WT) and DA−/− mice in this study were 3- to 6-month-old male and female hybrids of 129/SvEv × C57BL/6J. Because no significant differences between heterozygous and WT mice were observed in any of the parameters measured, they were grouped and designated as WT mice. Mice were housed under standard vivarium conditions and maintained on a 12-hr light/dark cycle with the lights on at 07:00. For routine maintenance of mutants, daily injections of 50 mg/kg L-DOPA were administered to DA−/− mice between 12:00 and 16:00. The genotypes of all mice were assessed by Southern blot analysis.

Activity Measurements. Transparent Plexiglas cages (40 × 20 × 20 cm) were placed on a rack equipped with infrared photobeams (San Diego Instruments) to measure the ambulatory activity of

Abbreviations: DA, dopamine; DA−/−, dopamine-deficient; L-DOPA, 3,4-dihyroxy-L-phenylalanine; 6-OHDA, 6-hydroxydopamine; WT, wild type.

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the mice. The number of consecutive beam-breaks that occurred each hour was measured and converted to meters by using the distance between beams (8.8 cm) as a conversion factor.

Neurological and Sensorimotor Examinations. WT (n = 10) and DA−/− (n = 10) mice were subjected to 13 neurological tests and 1 activation test (forced swim) that had previously been used to assess the capacities of 6-OHDA-lesioned rats (21). Catalepsy and biting strength tests were modified to make them suitable for mice. Catalepsy and akinesia tests were performed in the home cage. Mice did not respond to the tactile or noxious stimuli tests designed for rats; these tests were therefore omitted. Tests were performed at 1 and 24 hr after an L-DOPA injection, and mice were scored on a scale of 0 (poor) to 4 (normal).

Food Intake Measurements and Videotaping of Feeding Behavior. Fresh food pellets were placed in the bottom of the cage each day and water was available from 15- or 50-ml bottles equipped with spouts. The amount of food consumed was quantified by weighing the food pellets before and after each test interval and the difference was recorded. Water intake was quantified by weighing bottles in the same fashion. To keep DA−/− mice alive without L-DOPA treatment, Ensure Plus (Abbott Labs) was dispensed in small drops with a syringe and a blunt 30-gauge needle in front of the mouth of hand-held DA−/− mice (n = 3), beginning at postnatal day 21. Mice were routinely maintained on breeder chow (Purina 5015), which contains 11% fat and yields 4.35 kcal/g. For some experiments, we used a standard low-fat chow (Harlan 8604) with 3.93 kcal/g, or a high-fat, high-sucrose, soft palatable chow (Research Diets, D12331) with 5.56 kcal/g. For videotaping of feeding behavior, WT (n = 3) and DA−/− (n = 3) mice were fed the high-fat, high-sucrose, soft, palatable chow for 2 weeks. Food was removed on day 15, and 30 hr later, cage tops were carefully removed and a cup filled with the same type of chow was placed on the bottom of the cage. Reaction of the mice was videotaped for 90 min and the amount of food consumed was determined as described above.

Drug Administration. Drugs were prepared in 10 mM sodium phosphate, 150 mM NaCl, pH = 7.5 (PBS) or 0.9% NaCl. Solutions of L-DOPA (Sigma) at 1.5 mg/ml and the methyl ester of L-DOPA (Research Biochemicals International) at 3 mg/ml were prepared in PBS containing 2.5 mg/ml ascorbic acid (Sigma). Carbidopa (Sigma) was added to L-DOPA solutions at concentrations that allowed for delivery of either 12.5 or 25 mg/kg carbidopa with the standard 50-mg/kg dose of L-DOPA. Amphetamine (Sigma), SCH 23390 (Research Biochemicals International), and haloperidol (McNeil Pharmaceutical) were prepared in 0.9% saline. All drug solutions were delivered via intraperitoneal injection in a volume that did not exceed 1 ml.

D1- and D2-Receptor Antagonist Studies. Mice were placed in activity chambers for at least 12 hr before treatment with 0.9% saline or DA receptor antagonists (SCH 23390 and haloperidol); beam breaks were recorded over 4 hr. The DA−/− mice were treated with 50 mg/kg L-DOPA either 2 or 26 hr before antagonist treatment, such that activity measurements were performed 2 to 6 and 26 to 30 hr after L-DOPA administration.

Monoamine Measurements. The brains of the CO_2-asphyxiated, WT and DA−/− mice were removed and immediately placed in ice-cold PBS for 2 min. Dissections were performed on a glass plate chilled to 4°C, and structures of interest were frozen on dry ice in microcentrifuge tubes and stored at −70°C until analysis. Monoamine measurements were performed as described (22).

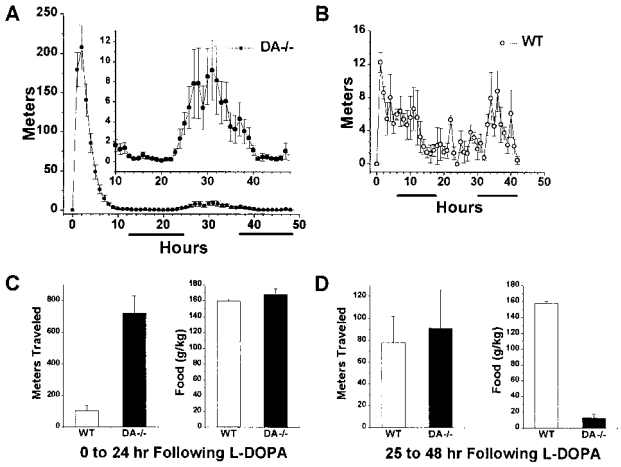

Fig. 1. DA−/− mice exhibit hyperactivity immediately after L-DOPA treatment, and display a second wave of activity that occurs 30 hr after treatment. Ambulatory activity of DA−/− mice (*A*; n = 17] and WT mice (*B*; n = 8). (*C* and *D*) Black bars represent the dark cycle. Total distance traveled and amount of food consumed by DA−/− and WT mice in the first 24-hr period (*C*) and second 24-hr period (*D*) after L-DOPA treatment.

Results

Locomotor Activity of DA−/− Mice After Injection of L-DOPA. Daily administration of 50 mg/kg L-DOPA into DA−/− mice induced locomotor activity that greatly exceeded that of L-DOPA-treated WT mice for 6 to 9 hr; by 24 hr, however, the DA−/− mice had become hypoactive (Fig. 1*A*). Because the DA−/− mice remained hyperactive for an entire day when injected every 4 hr with 100 mg/kg L-DOPA, and they traveled up to 3000 meters/day (data not shown), the decline in activity after the first few hours was not caused by fatigue. The overall distance traveled by DA−/− mice during the first 24 hr after treatment was 7-fold greater than that of WT mice (Fig. 1*C*). An unexpected second wave of activity occurred between 24 and 40 hr after the previous L-DOPA treatment (Fig. 1*A*, *Inset*). The mean distances traveled by DA−/− and WT mice between 24 and 48 hr after an L-DOPA injection were equivalent (Fig. 1*D*); however, during this period of activity DA−/− mice typically displayed a hunched appearance, walked with an awkward gait, and performed poorly on rotarod and pole tests (23).

The distance traveled by DA−/− mice increased marginally as the dose of L-DOPA was increased to 20 mg/kg, then steadily increased to reach a plateau between 40 and 100 mg/kg L-DOPA (Fig. 2*A*). None of these dosages of L-DOPA had any effect on WT mice.

Neurological and Sensorimotor Examination of WT and DA−/− Mice. Mice performed well on many of the neurological tests (Table 1). DA−/− mice scored 486 (1 hr after L-DOPA), but only 379 (24 hr after L-DOPA) out of a possible 520 points, compared to 495 for WT mice. DA−/− mice tested 24 hr after L-DOPA treatment displayed greater one- and four-limb akinesia and forelimb catalepsy than did WT mice (Table 1). When tested 1 hr after L-DOPA treatment, the DA−/− mice were deficient in the paw-pinch assay. The performance of the DA−/− mice in the forced-swim test 24 hr after L-DOPA treatment was equivalent to that of the WT mice.

Food Consumption of DA−/− Mice in Response to L-DOPA. DA−/− mice can be maintained for at least 1.5 yr with daily administration of 50 mg/kg L-DOPA if they are fed breeder chow (4.35 kcal/g); standard chow (3.93 kcal/g), however, is inadequate for survival. With treated daily with L-DOPA injections at 25 mg/kg,

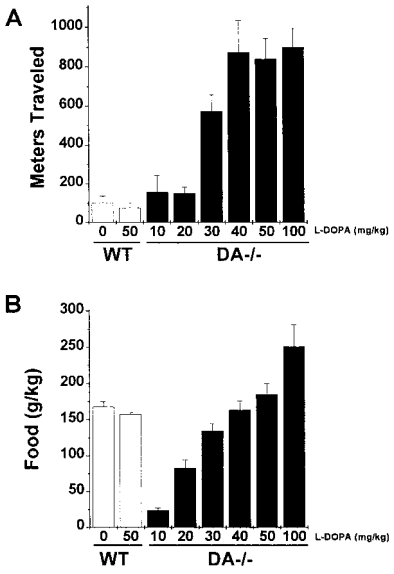

Fig. 2. Low doses of L-DOPA cause hyperactivity in DA−/− mice. Individually housed DA−/− mice during the 9-hr period after administration of 10, 20, 30, 40, or 50 mg/kg or 100 mg/kg L-DOPA methyl ester were used to generate a dose-response curve. Various doses of L-DOPA were administered 24 hr after their last L-DOPA injection. (*A*) Ambulatory activity of WT ($n = 8$) and DA−/− mice ($n = 8$). (*B*) Food consumed by WT ($n = 8$) and DA−/− mice ($n = 8$).

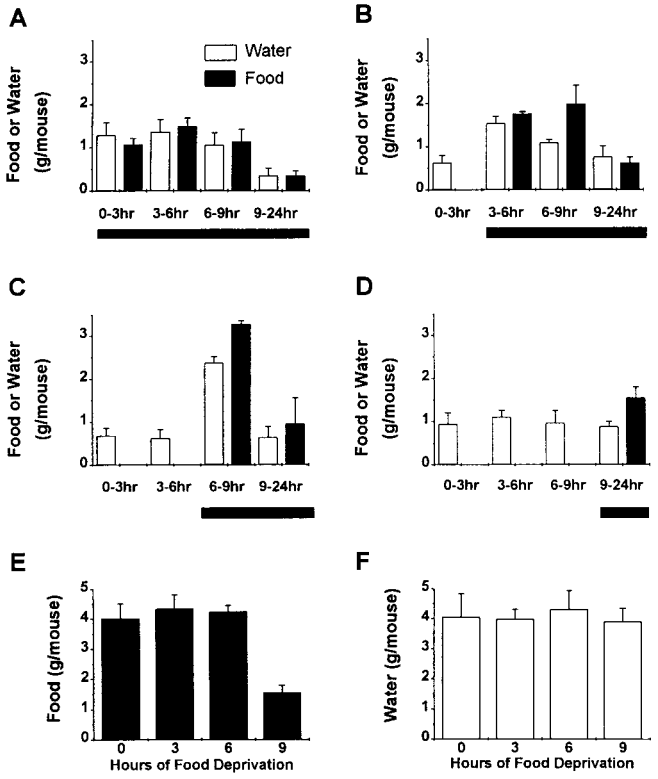

Fig. 3. Food deprivation identifies the minimal amount of DA required to sustain food consumption in DA−/− mice. Mice ($n = 15$) housed in groups of three were treated with 50 mg/kg L-DOPA, returned to their home cages, and food was added after 3, 6, or 9 hr of restriction. Water was available ad libitum during the food restriction studies. Amount of food and water consumed after 0 (*A*), 3 (*B*), 6 (*C*), or 9 (*D*) hr of food deprivation is shown. Black horizontal lines represent the time when food was present in the cage. (*E*) Total amount of food consumed when mice were food-deprived after L-DOPA treatment. (*F*) Water consumed by food-deprived DA−/− mice.

the DA−/− mice lost weight and would have succumbed if this regimen had continued.

DA−/− mice housed together consumed about 30% of their total daily food and water during each of the first three 3-hr periods, and the remaining 10% was consumed during the next 15 hr (Fig. 3*A*). Water and food consumption by the two groups were comparable during the 24 hr. The total amounts of food consumed by DA−/− and WT mice during the first 24 hr after L-DOPA treatment were similar; however, during the next 24 hr, the DA−/− mice ate only 10% as much food as did the WT mice (Fig. 1 *C* and *D*). Food intake was proportional to the L-DOPA dose up to 100 mg/kg (Fig. 2*B*).

In another experiment, DA−/− mice were injected with L-DOPA but food was unavailable until 3, 6, or 9 hr later; water was available all the time. When food was restored 3 or 6 hr after the L-DOPA injection, the DA−/− mice compensated by eating more during the next 3-hr period (Fig. 3 *B* and *C*); by 24 hr, they had consumed the same amount of food as under ad libitum conditions (Fig. 3*E*). However, if food was restored at 9 hr, the DA−/− mice ate only 35% of the daily requirement (Fig. 3 *D* and *E*), but they consumed normal amounts of water even when food consumption fell below normal (Fig. 3 *E* and *F*). Although food and water consumption normally occurred in parallel (Fig. 3*A*), they can be dissociated (Fig. 3 *B–D*).

Table 1. Neurological testing of WT and DA−/− mice

	Test score		
	WT	DA−/− mice	
Test	24 hr	1 hr	24 hr
Screen paw placement	40	40	40
One-limb akinesia	39	40	14***
Four-limb akinesia	40	40	9***
Turning on a tilted screen	35	29	7**
Forelimb catalepsy	40	39	14***
Visual placing	36	39	32
Orientation to paw pinch	40	33*	37
Auditory response	40	40	40
Vestibular response	40	40	40
Splay posture	40	40	40
Biting strength	25	26	26
Righting reflex	40	40	40
Hind-limb extensor rigidity	40	40	40
Total	495	486	379

Neurological tests were performed on WT and DA−/− mice 1 and 24 hr after L-DOPA treatment. No differences were observed in WT mice at 1 and 24 hr. Maximum possible score for each test was 40 (4 × 10 mice). Significance was assessed by standard ANOVA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with WT mice.

The Feeding Behavior of DA−/− Mice. Hand-held, young DA−/− pups that had never been injected with L-DOPA would lick and swallow small drops of a liquid diet placed by their mouth. With frequent feeding by this method, the mice ate enough to maintain body weight for a week without L-DOPA treatment; however, if left with their mother, they would have died.

When presented with a palatable, high-sucrose, high-fat diet after a 30-hr fast, the adult DA−/− and WT mice approached, sniffed, and began to consume the food; but consumption by the DA−/− mice was short-lived compared with that of the WT mice. After 90 min, most of the DA−/− mice were inactive or sleeping, whereas WT mice were still moving about the cage and occasionally eating. The amount of food eaten by the DA−/−

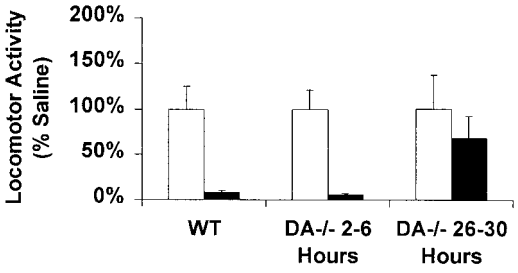

Fig. 4. DA receptor antagonists do not block the second wave of locomotion displayed by DA−/− mice. Ambulatory activity of WT and DA−/− mice treated with 0.9% saline (open bars) or a mixture of 0.1 mg/kg SCH 23390 and 2 mg/kg haloperidol (closed bars) shown as percent of saline-elicited activity. Meters traveled ± SEM: WT saline = 24.6 ± 6.2; DA−/− saline (2–6 hr) = 281.1 ± 59.3; and DA−/− saline (26–30 hr) = 17.9 ± 6.7.

mice was only 25% that of the WT mice during this 90-min period.

The Second Wave of Activity. The second wave of activity may have been caused by the release of residual DA. To test this possibility, a mixture of D1- and D2-receptor antagonists (SCH 23390 and haloperidol, respectively) was administered during the second wave of activity and locomotion was monitored. The DA-receptor antagonists reduced locomotion by more than 90% in WT and DA−/− mice during the first wave of activity (Fig. 4). The same mixture of antagonists had no significant effect on the locomotion of DA−/− mice during the second wave of activity (Fig. 4).

Amphetamine stimulates locomotion in rodents by inducing the release of DA from dopaminergic terminals (24, 25). Administration of 5 mg/kg amphetamine to DA−/− mice 19 hr after their last treatment with L-DOPA stimulated ambulatory activity. However, the response of DA−/− mice was completed within 1 hr, compared with 2 hr for WT mice (Fig. 5*A*). The WT mice responded similarly to a second dose of amphetamine given 2 hr after the first dose, whereas the DA−/− mice were completely unresponsive to a second dose (Fig. 5*A*). The locomotor response of DA−/− mice to amphetamine was completely blocked by a combination of D1- and D2-receptor antagonists (data not shown). Fig. 5*B* demonstrates that a second wave of activity occurred on schedule even when residual DA was depleted by amphetamine.

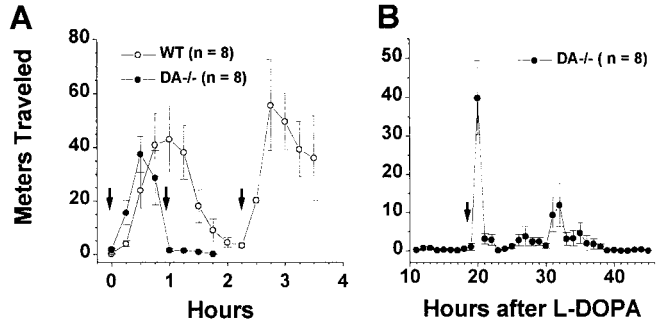

Fig. 5. Amphetamine induces locomotor behavior in DA−/− mice, but amphetamine treatment does not block the second wave of activity displayed by DA −/− mice. (*A*) Ambulatory activity of WT and DA−/− mice given two consecutive injections of 5 mg/kg amphetamine. Arrows indicate times of amphetamine administration. (*B*) Amphetamine (5 mg/kg) was administered 19 hr after the L-DOPA injection and activity was monitored for an additional 36 hr. Arrow indicates time of amphetamine injection.

Table 2. Catecholamine content in WT and DA−/− mice

Group	Brain region	*n*	Time after treatment, hr	L-DOPA, mg/kg	DA, ng/mg protein
WT	Striatum	7	0	Naïve	108.05 ± 12.02
		10	3	50	91.14 ± 7.98
		5	30	50	89.14 ± 10.15
DA−/−		5	0	Naïve	0.48 ± 0.05
		10	3	50	8.31 ± 1.46*,**
		4	9	50	3.47 ± 0.54*
		5	30	50	0.50 ± 0.05*
		4	3	100†	25.79 ± 6.04*,**
		8	3	50 + CD	101.86 ± 18.77**
WT	Thalamus	10	3	50	12.29 ± 1.87
		5	30	50	13.29 ± 2.52
DA−/−		12	3	50	3.13 ± 0.55*
		5	30	50	0.98 ± 0.16*
		8	3	50 + CD	39.28 ± 2.48*,**
WT	Midbrain	7	0	Naïve	2.89 ± 0.35
		9	3	50	2.37 ± 1.38
		6	30	50	2.15 ± 0.87
DA−/−		5	0	Naïve	0.47 ± 0.07*
		10	3	50	1.61 ± 0.29
		4	9	50	0.81 ± 0.05
		5	30	50	0.63 ± 0.13*
		4	3	100†	1.17 ± 0.16
		4	3	50 + CD	8.71 ± 1.03*,**

WT and DA−/− mice were injected with L-DOPA, L-DOPA methy ester (†), or L-DOPA + 25 mg/kg carbidopa (CD), and animals were sacrificed at 3, 6, 9, or 30 hr after treatment. Naïve mice did not receive injection of L-DOPA and were sacrificed when 17 to 20 days old. Brains from all mice were removed and dissected and catecholamine concentrations were measured with HPLC and electrochemical detection. Significance was assessed by standard two-tailed *t*-test analysis. *, $P < 0.05$ compared with WT mice; **, $P < 0.05$ compared with naïve DA−/− mice.

Brain DA Content Is Only Partially Restored by L-DOPA. At the peak of activity, 3 hr after the standard dose of 50 mg/kg L-DOPA, the highest DA content was found in the striatum, but it was only 9.1% of the WT levels (Table 2). At 9 hr, when there was insufficient DA to support normal food consumption (Fig. 3*E*), DA content was 3.8% of normal; at 30 hr, DA levels were <1% of normal (Table 2). The ratio of DA to norepinephrine in the striatum of naïve DA−/− mice never injected with L-DOPA was 0.08, not significantly different from the ratio obtained at 30 hr (0.1). This value is also similar to the ratio observed in brain regions that contain few dopaminergic projections (e.g., cerebellum) and several peripheral tissues (22). Administration of 100 mg/kg of the L-DOPA methyl ester raised brain DA content in the striatum (28.2% of normal) but not in the midbrain (Table 2). Norepinephrine and serotonin levels were normal in DA−/− mice 3 and 30 hr after L-DOPA treatment (data not shown).

Carbidopa Elevates Brain DA Concentrations and Induces Stereotypy. To achieve higher levels of L-DOPA in the brain, peripheral L-aromatic amino acid decarboxylase activity was inhibited by coinjecting carbidopa. Administration of L-DOPA and carbidopa restored DA content within the striatum to normal levels and to more than normal levels in the midbrain (Table 2). In addition, the concentration of the primary DA metabolite, dihydroxyphenylacetic acid (DOPAC), was elevated in all brain

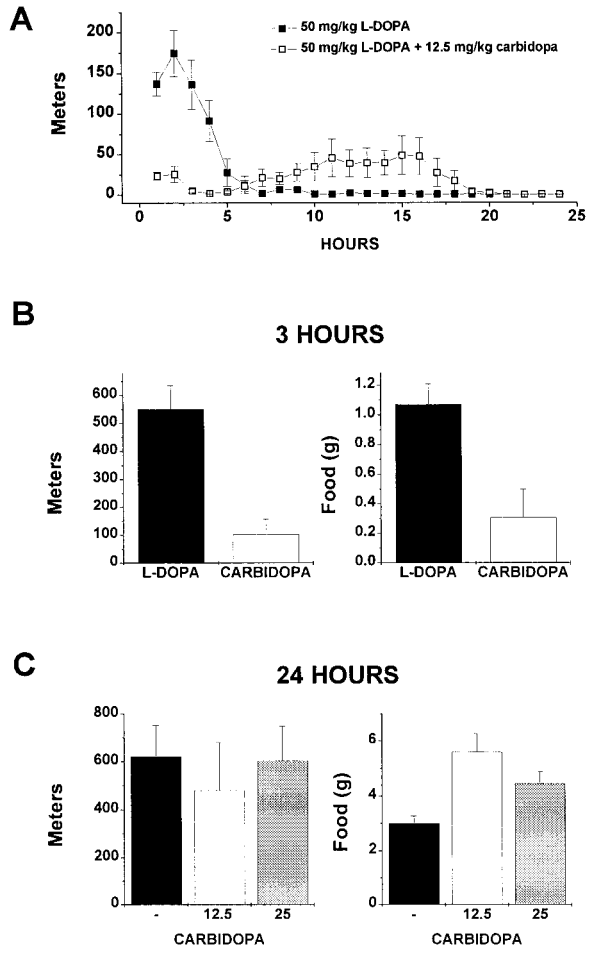

Fig. 6. Simultaneous carbidopa and L-DOPA treatment induces stereotypy in DA−/− mice. (*A*) Ambulatory activity of DA −/− mice ($n = 8$) treated with 50 mg/kg L-DOPA (■) or a mixture of 50 mg/kg L-DOPA and 12.5 mg/kg carbidopa (□) parse by 1-hr intervals. (*B*) Total meters traveled and food consumed by DA−/− mice in the first 3 hr after injection of 50 mg/kg L-DOPA alone (black bars) or 50 mg/kg L-DOPA and 12.5 mg/kg carbidopa (open bars). (*C*) Total meters traveled and food consumed by DA−/− mice in 24 hr when treated with 50 mg/kg L-DOPA alone (black bars), a mixture of 50 mg/kg L-DOPA and 12.5 mg/kg (open bars), or 50 mg/kg L-DOPA and 25 mg/kg carbidopa (gray bars).

regions, suggesting that DA turnover was elevated (data not shown). When carbidopa at 12.5 or 25 mg/kg was given with the standard 50 mg/kg dose of L-DOPA, it produced a biphasic activity profile, with a lull in ambulatory activity between 1 and 5 hr (Fig. 6*A*). During this lull, DA−/− mice typically sat upright with their paws together and engaged in intense stereotypic behavior that included licking and chewing of paws and occasionally self-mutilation. This treatment produced less locomotor activity and feeding during the 3 hr after treatment than that achieved by L-DOPA alone (Fig. 6*B*), but activity resumed and persisted much longer, such that during the 24 hr, these mice traveled a distance similar to that when they were given L-DOPA alone. The mice also ate more food with either dose of carbidopa (Fig. 6*C*). WT mice treated with L-DOPA and either dose of carbidopa became inactive and did not exhibit stereotypy.

Discussion

There is extensive literature implicating DA in movement, sensorimotor function, and feeding. DA−/− mice are unable to synthesize DA in dopaminergic terminals without pharmacological intervention or gene therapy, and consequently, when depleted of DA, they display defects in many of these activities. When DA−/− mice are treated with L-DOPA, the concentration of DA restored in the brain is only a fraction of WT levels (Table 2), but they manifest locomotor activity that reaches 200 meters/hr, about 20 times that of WT mice (Fig. 1*A*). Food consumption is normal with this routine L-DOPA treatment (Fig. 1*C*). These results demonstrate that DA−/− mice are supersensitive to brain DA. This supersensitivity to DA does not appear to be caused by differences in the number of DA receptors or in the DA transporter, as measured by ligand binding, suggesting that it resides in more efficient coupling of DA receptors to the signal transduction (unpublished observations).

Adult 6-OHDA-lesioned rats fail to move, eat, or drink for several days after surgery, but with assistance, the rats will progress through several stages of behavioral recovery and eventually regain the ability to perform these behaviors (26). In the early stages of recovery, a phenomenon called "paradoxical kinesia" can be elicited in which activity and feeding can be restored for a short time by presentation of novel stimuli (21, 27). DA−/− mice resemble rats in this early stage; they become hypoactive, aphagic, and adipsic, and "paradoxical kinesia" can be induced 24 hr after L-DOPA treatment. Even without stimulation, DA−/− mice undergo a DA-independent wave of motor activity 24–48 hr after L-DOPA treatment (Figs. 1 and 4). During this second wave of activity, the DA−/− mice approach, sniff, chew, and swallow food, but they consume only a fraction of that needed for sustenance. In the rat 6-OHDA model, there is gradual compensation for the feeding deficit when the lesions are performed in adults, and no deficits are observed after neonatal lesions; in both cases, brain DA levels are reported as $<$ 2% of normal. Although increases in serotonin have been observed (28, 29), normal feeding continues to depend on DA; i.e., a second 6-OHDA treatment or inhibition of tyrosine hydroxylase activity reinstates aphagia (6). We observe insufficient feeding in DA−/− mice when DA falls below 3.8% of normal (9 hr after L-DOPA administration), serotonin levels are unaltered, and compensation is not observed even after a year of daily L-DOPA treatments.

Responses of DA−/− mice in 9 of 13 tests that involve external stimuli, as well as the forced-swim test, were normal and unaffected by L-DOPA treatment. However, their responses in 4 tests that involve voluntary or coordinated movement were impaired 24 hr after L-DOPA but were restored 1 hr after L-DOPA treatment (Table 1). This finding is in agreement with the inability of DA−/− mice to perform other motor tasks that require coordinated movement (pole and rotarod test) when DA is depleted (14, 20). Overall, DA −/− mice performed at 76.6% (1 hr after L-DOPA) and 98.2% (24 hr after L-DOPA) of WT mice on these tests. DA−/− mice ambulate normal distances during the second 24 hr period after L-DOPA treatment without consuming the normal amount of food. However, they ate normally for a short time when presented with palatable food during that period. They could also be kept alive for over a week without L-DOPA treatment by hand-feeding them a liquid diet. These observations indicate that DA−/− mice can execute behaviors required for seeking and ingesting food; however, without L-DOPA treatment they will die of starvation even with easy access to palatable food. Likewise, DA−/− pups with a nursing mother will not suckle enough to sustain themselves.

It is noteworthy that adult rats with severe 6-OHDA lesions were maintained by gastric feeding rather than pharmacologically. In that model, dopaminergic terminals are almost completely destroyed and those that remain probably manifest maximal activity; hence DA release cannot be enhanced by administering L-DOPA. We have found it difficult to restore normal feeding (or other behaviors) of DA−/− mice with

direct-acting DA agonists and the same may be true for lesioned rats. Thus, activity-dependent release of DA from dopaminergic terminals (perhaps along with other neuromodulators) may be necessary for normal feeding.

An optimal level of dopaminergic signaling may be required for the mice to engage in normal behaviors. Peripherally administered DA agonists or antagonists inhibit feeding in both WT and partially lesioned rats, suggesting that acute elevation or dampening of DA signaling adversely affects rat feeding behavior (8, 30). DA−/− mice also have an optimal range of brain DA levels (3.5–25% of normal) that can support normal feeding. When DA falls below that range, the mice do not consume adequate amounts of food; when DA rises above that range, intense stereotypic behaviors interfere with feeding. Elevation of brain DA concentrations to WT levels causes DA−/− mice to engage in intense stereotypy and forego eating for several hours; but when DA levels begin to decline, they ambulate and feed as when injected with L-DOPA alone (Fig. 6 *A–C*). The maximal locomotor response observed with this treatment does not reach the magnitude obtained with L-DOPA treatment alone, but activity and feeding are prolonged.

What neuromodulator(s) induce the second wave of locomotor activity? We suspect that the second wave of activity is the consequence of a circadian input. Considering that dopaminergic neurons are intact in this model, the simplest suggestion is that these neurons normally liberate neuromodulators (in addition to DA) that contribute to voluntary locomotion. In the absence of DA, these other neuromodulators may continue to stimulate locomotion in response to circadian inputs. Neuromodulators/neurotransmitters, such as cholecystokinin, neurotensin, ATP, adenosine, or glutamate are candidates (31–35). Alternatively, the circadian activity may be completely independent of inputs from dopaminergic neurons.

The DA−/− mice reveal that DA is essential for normal feeding. The same conclusion was reached many years ago by several investigators studying rats with 6-OHDA lesions; however, the insult produced by destroying neurons greatly exceeds that resulting from the removal of a single enzyme. Thus, it is remarkable that the 6-OHDA lesions and genetic model are so similar, despite the fact that dopaminergic neurons produce many neuromodulators in addition to DA.

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