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Mapping of globus pallidus and ventral pallidum lesions that produce hyperkinetic treading

Howard C. Cromwell, Kent C. Berridge *

Department of Psychology, The University of Michigan, 1103 E. Huron Street, Ann Arbor, MI 48104, USA

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Abstract

The purpose of this study was to identify sites where striatopallidal lesions produce two distinct sensory-triggered hyperkinetic syndromes: (1) exaggerated forelimb treading alone to oral taste infusions and (2) sensorimotor exaggerated treading plus enhanced aversive reactions to taste infusions. The behavioral characteristics of these syndromes have been described previously (Berridge, K.C. and Cromwell, H.C., *Behav. Neurosci.*, 104 (1990) 778–795). Bilateral excitotoxin lesions were made using quinolinic acid (10 μg in 1 μl) in the caudate/putamen, nucleus accumbens, globus pallidus or ventral pallidum/substantia innominata. In order to identify the precise center, borders, severity and size of lesion sites that caused these hyperkinetic treading syndromes, neuron counts (modified fractionator technique) and glial fibrillary acidic protein immunoreactivity (GFAP-IR) densitometry were used in a stereological mapping analysis. The site of lesions that produced the hyperkinetic treading syndrome without enhanced aversion was found to be restricted to the globus pallidus (GP). Damage exceeding 60% neuron loss bilaterally within a 0.8 × 1.0 × 1.0 mm subregion of the ventromedial GP produced this syndrome. The site of lesions that produced the combined syndrome of hyperkinetic treading and aversive enhancement was ventral to the globus pallidus, within the ventral pallidum/substantia innominata (VP/SI). Damage exceeding 70% neuron loss bilaterally within a 1.0 × 0.5 × 1.0 mm diameter subregion of the ventromedial ventral pallidum/substantia innominata produced this syndrome. This subterritory was located immediately lateral to the border of the lateral hypothalamus. Bilateral lesions to the caudate/putamen or nucleus accumbens did not produce either hyperkinetic treading syndrome. These results are discussed in terms of the connectivity of the ventral pallidal/substantia innominata and globus pallidus regions and in terms of neuropathological models of hyperkinetic disorders.

Keywords: Striatopallidal; Globus pallidus; Ventral pallidum; Hyperkinesia; Quinolinic acid; Lesion mapping

1. Introduction

Hyperkinetic movements of Huntington's disease [73], Gilles de la Tourette's syndrome, and tardive dyskinesia are thought to originate from disturbances in neuronal activity of the striatopallidal system [1,6,25,68,75]. Pathological movements of these disorders can range from brief, simple motor tics (defined as "stereotyped jerks of isolated muscles of the upper parts of the body"; see Thompson et al. [71] p. 214) to choreiform sequences (defined as "brisk, graceful series of successive, involuntary movements of considerable complexity which resemble fragments of purposeful voluntary movements"; see Carpenter [17] p. 253). Examples of the former include an abrupt shrug of the shoulder or rapid forearm extension while the latter could involve a combination of forelimb reaches intertwined with finger flexion or hand grasping motions.

Most animal models of hyperkinesia only partially replicate the characteristics of any of these disorders. In humans, hyperkinetic movements are often spontaneous after damage to particular brain regions, yet in many animal models hyperkinetic movements appear only after the administration of drugs. For example, Crossman and colleagues have produced hyperkinetic movements in the monkey by injecting bicuculline, a GABA receptor antagonist, into the lateral globus pallidus [20,42]. Movements analogous to chorea are seen in monkeys given levodopa or apomorphine after stri-

* Corresponding author. Neuroscience Laboratory Building, The University of Michigan, 1103 E. Huron Street, Ann Arbor, MI 48104, USA. Fax: (1) (313) 936-2690.

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atal lesions [37,44]. In the rat, chronic systemic injections of 3,3-iminodipropionitrile, a neurotoxin, have produced choreiform and athetoid head and neck movements [67], and intracaudate injections of a GABA antagonist combined with sensorimotor cortex damage have produced rhythmic jerking of the limbs [70]. In a few studies of non-human primates, spontaneous hyperkinesia without drug administration has been produced. Denny-Brown reported a 'wing-beating' motion in monkeys with putamen lesions [22], and Carpenter has reported hyperkinetic movements following bilateral pallidal damage in the macaque monkey [16]

Recently, a novel hyperkinetic movement pattern has been documented in the rat, which occurs after striatopallidal lesions [11]. This hyperkinetic movement pattern is perhaps the first to be reported for rodents that occurs without need of pharmacological manipulation. The movement pattern resembles both the hyperkinetic movements of Huntington's disease and of Tourette's syndrome in several respects [10,11]. In the rat, the hyperkinetic movement is triggered by oral sensory input not unlike the sensory-triggered motor tics observed in certain cases of Tourette's syndrome [14,46]. The hyperkinetic movement in rats is an exaggerated paw treading: a rapid, vigorous and rhythmic forelimb extension followed by retraction. Hyperkinetic treading is an exaggeration of normal but infrequent paw treading seen in intact rats in response to oral infusions of highly bitter-tasting solutions. Normal paw-treading described by Grill and Norgren [26] is composed of smaller forelimb movements ranging in amplitude from 1–5 mm and lasting as a series of movements typically 1–5 s.

The pathological exaggeration of treading produced by striatopallidal lesions is manifest by an increase in incidence, amplitude, and duration of the movement pattern compared to normal rats. After large excitotoxin lesions of the posterior corpus striatum and pallidal region, 100% of rats show exaggerated treading to oral infusions of quinine (as opposed to only 10% of normal rats that show ordinary treading). The amplitude of the movements is increased 5 to 10 times (ranging from 1 cm to 3 cm in each cycle for the hyperkinetic form) and the duration of a bout is increased 3 to 10 times (ranging from 12 to 60 s of a 1 min trial) [10,11].

A peculiar feature of some hyperkinetic disorders in humans is that the emotional state of the patient may affect the expression of the hyperkinetic movement produced by the disease [15]. For example, choreic movements become more frequent and greater in amplitude upon increased arousal or stress [45]. When a psychological 'load' is added to a motor task for patients with choreiform disorder, the choreic movements become more pronounced compared to the choreic movements seen in the same motor task ordinarily [57]. Hyperkinetic treading by the rat with a large striatopallidal lesion is similar in that it also is regulated by emotional state. In particular it is enhanced by certain aversive states. For example, some rats with large lesions of the striatopallidal system display the hyperkinetic treading to only bitter 'aversive' tasting solutions and not sweet 'palatable' tasting solutions. These rats can be converted to show the hyperkinetic treading to sweet 'palatable' tastes by associatively pairing these sweet solutions (sucrose) with LiCl-induced illness [11]. This result suggests that motivational aversion and sensorimotor pathology interact to control the final expression of pathological treading by rats after striatopallidal damage [11,19].

Hyperkinetic treading in the rat can occur in two distinct forms or syndromes after striatopallidal lesions depending apparently on properties of the lesion such as location, size or severity of neuron loss [11]. In the first syndrome (treading only or 'T syndrome'), rats emit hyperkinetic treading only to bitter or other unpalatable tastes, and are essentially normal in locomotion and feeding behavior. In the second syndrome (enhanced aversion plus treading or 'A + T syndrome'), rats emit hyperkinetic treading to all tastes and show aversive reactions (e.g. gapes, head-shakes, chin-rubs, and forelimb-flails) to all tastes, even to normally palatable sucrose. The exact location and boundary of the striatopallidal site or sites where damage produces hyperkinetic treading has not previously been identified. Nor has it been shown how the two syndromes differ in terms of their neural damage. The present study was conducted in order to identify the crucial properties of the lesion that determine which syndrome is produced. Procedures were employed to measure the size, severity, and the neuroanatomical boundaries of the sites for the two syndromes. A novel neuron counting technique, a modification of Gundersen et al.'s 'fractionator' procedure [33] and immunocytochemical staining for glial fibrillary acidic protein were used to precisely identify these lesion sites.

2. Materials and methods

2.1. Subjects

Subjects were 47 male and 56 female Sprague–Dawley rats (Charles-River) weighing 300 ± 50 g at the beginning of the experiment. They were housed singly under a 12:12 light:dark cycle. Water and food were freely available.

2.2. Surgery

Rats were anesthetized using first halothane (within a desiccator chamber) and then methoxy-fluorane given

through an air anesthesia system for small animals [49]. Atropine sulfate (3 mg/kg, i.p.) and diazepam (8 mg/kg) were given approximately 30 min prior to neurotoxin injection. Bilateral lesions were made using quinolinic acid (10 μg in 1 μl). This neurotoxin has been suggested to play a role in the natural production of certain hyperkinetic disorders such as Huntington's disease in humans [13]. Most lesions were focused on posterior and ventral striatopallidal structures, since previous studies have indicated that lesions of these structures may have a special role in exaggerated treading [11,19]. Lesions intended to damage the ventral striatum or nucleus accumbens were centered at the coordinates: AP +1.7 mm anterior to bregma; L $\pm$1.5 mm lateral to bregma; V $-$7.4 mm below the skull ($n = 8$ rats). Lesions intended to damage the neostriatum or caudate nucleus and putamen (CPU) were aimed into four different quadrants. These included the dorsolateral quadrant: AP +1.0 mm; L $\pm$3.7 mm; and V $-$4.5 mm ($n = 8$ rats), the dorsomedial quadrant: AP +1.0 mm; L $\pm$1.6 mm; V $-$4.2 mm ($n = 8$), the ventromedial quadrant: AP +1.0 mm; L $\pm$1.7 mm; V $-$5.6 mm ($n = 4$) or the ventrolateral quadrant: AP +1.0 mm; L $\pm$3.6 mm; V $-$6.6 mm ($n = 8$). Lesions intended to damage the globus pallidus were centered at the coordinates: AP $-$1.0 mm; L $\pm$2.8 mm; V $-$7.5 mm ($n = 13$ rats). Lesions intended to damage the ventral pallidum/substantia innominata were centered at the coordinates: AP $-$1.0 mm; L $\pm$2.5 mm; V $-$8.0 mm ($n = 10$). The actual center of the lesions varied around these coordinates. The vehicle solution was always phosphate buffered saline (pH = 7.4). Sham operated controls received infusions of PBS alone (1 μl, nucleus accumbens, $n = 8$, CPU, $n = 28$, pallidal/SI, $n = 8$). Each infusion was made over a 3 min period, and the needle was left in place for an additional 5 min.

Each rat was implanted with bilateral chronic oral cannulae to allow for the infusion of the taste solutions into the mouth. These cannulae (heat-flared PE-50 tubing) [27] enter the mouth lateral to the first maxillary molar and exit the head near the dorsolateral boundary of the skull, where they are anchored with skull screws and acrylic cement.

Thirty min post-surgery, a second injection of diazepam (8 mg/kg) was given to reduce potential convulsive activity. Bicillin (30,000 units, i.m.) was given to reduce the chances of post-surgical infection. All rats were given 250 ml of cereal mash (commercial baby cereal mixed with water), 5 chow pellets and 40 ml of water per day (5–7 p.m. feeding). Aphagia and adipsia may follow striatopallidal neurotoxin injections [50]. Intake was monitored by counting the number of pellets consumed, the approximate amount of mash eaten (e.g. 50%), and the amount of water drank within a 24-h period. Rats were weighed daily. If a rat lost weight compared to his pre-surgical weight, then an intubation procedure was initiated. For each 5 g of weight lost, the rat was intubated with 12 ml of vitamin supplemented sweetened milk solution, up to 3 intubations per day. Rats were considered aphagic if neither chow nor pellets were eaten.

2.3. Behavioral observations

Sensory-triggered hyperkinetic treading and other taste reactions were videotaped, after an initial habituation trial, to oral infusions of sucrose (1 M) or quinine HCL (3×10^{-4}) in counterbalanced order. Sucrose elicits primarily ingestive responses and quinine elicits primarily aversive responses from normal rats. The initial trial (48 h post-surgery) used distilled water oral infusions for habituation.

A delivery tube was connected to the oral cannula, and the rat was placed in a transparent test chamber, which was suspended over an angled mirror. The mirror reflected a view of the rat's mouth and forepaws to the videocamera. Following a 5 min habituation period, 1 ml of the test solution was infused directly into the mouth over a 1 min period. If multiple stimuli were given in a single day, there was a two hour inter-test interval. Tests were run between 4 and 8 p.m. (i.e. shortly prior to lights out). For aphagic rats receiving intubations, taste trials were conducted at least 2 h after the last intubation.

2.4. Taste reactivity scoring

Videotapes were scored in slow motion (frame by frame to 1/10 normal speed) for the occurrence of exaggerated paw treading. A stringent criterion was used for the analysis of paw treading in order to avoid 'false positive' scoring of normal treading and ensure that only pathological exaggerated paw treading would be included. A unit of paw treading was required to be at least 5 continuous seconds of rhythmic forward and backward extension of the forepaws 180° out of phase, with the two paws extended at least 1 cm apart in every cycle, at a frequency of roughly 3.5 Hz.

Videotapes were also scored for the occurrence of aversive reactions and for positive hedonic reactions. Aversive actions include: gapes: large openings of the mouth and jaw lasting approximately 125 ms; chin-rubbing: bringing the chin in direct contact with the floor and projecting the body forward; face-washing: either a single wipe with the paws over the face or a bout of several wipes; forelimb flails: shaking of the forelimb back and forth; and rapid head-shaking. Paw treading was specifically excluded from the aversive scores to reduce artificial aversive score inflation.

The following positive reactions were scored: lateral tongue protrusions (nonrhythmic) lasting about 160 ms; rhythmic tongue protrusions: these protrude along the midline with a cycle length of roughly 160 ms; and paw-licking. Each action was scored and counted using criteria described in Grill and Norgren [26].

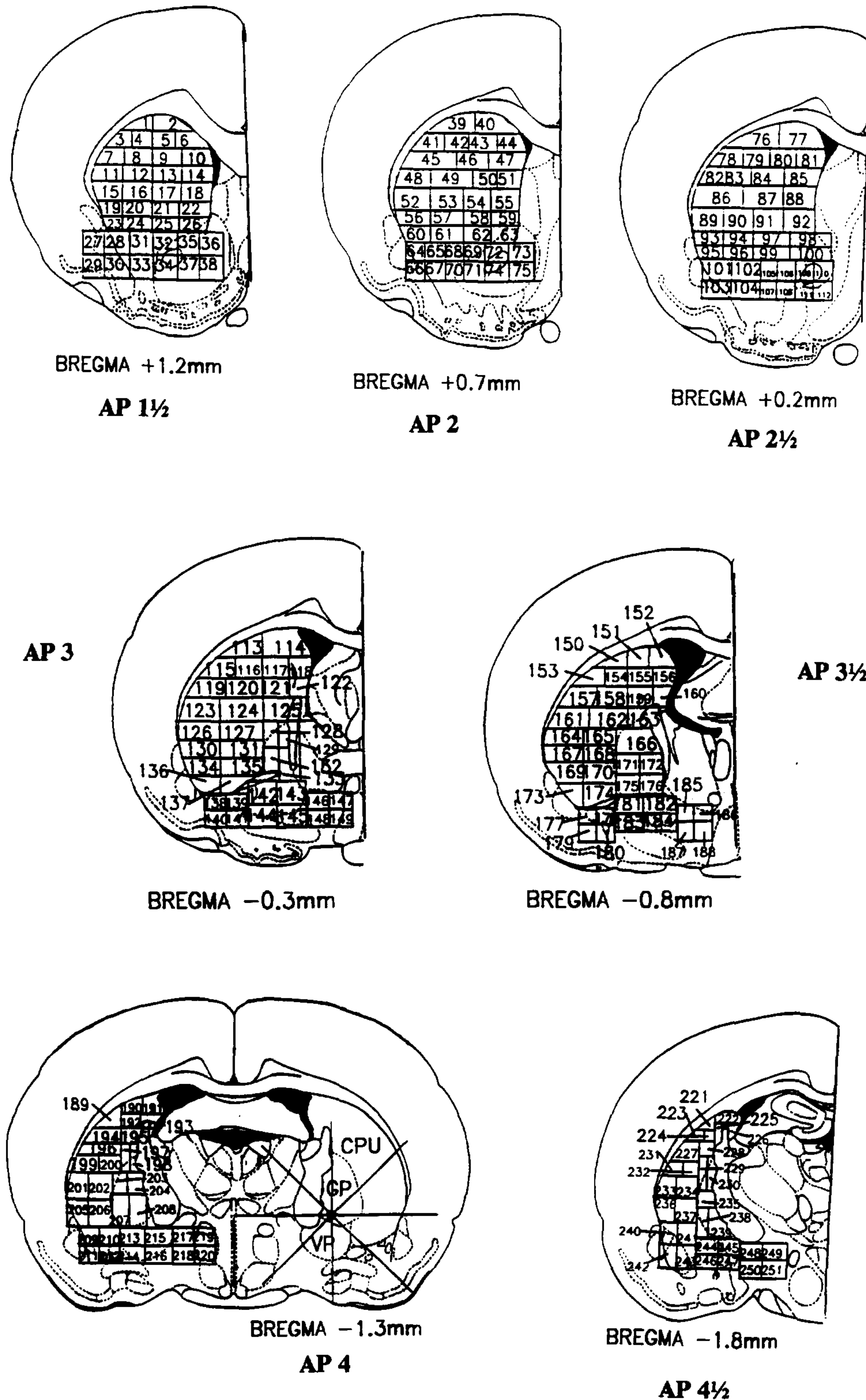

Fig. 1. Fraction zones. The 251 fraction zones used for the fractionator technique. Each fraction is labeled by its assigned number. Smaller fractions are positioned around the excitotoxin microinjection targets. Lower left: example of lesion boundary analysis along 8 radial arms from the center of a lesion. Neuron counts were continued along each arm until either the boundary of the lesion edge or the circumference was reached (2.5 mm from center, whichever came first.

2.5. Histology

At the conclusion of the behavioral testing, each rat was deeply anesthetized and intracardially perfused. Perfusions were completed with 0.9% saline followed by 10% formalin in PBS (pH = 7.4). Brains were removed and stored in a 30% sucrose solution (10% formalin). The brains were blocked, frozen, and sliced into 30 μm slices using a sliding microtome. Slices were mounted directly onto gelatin-coated slides for Cresyl violet staining. The slides were dipped in xylene and ethanol baths (70%, 95% and 100%) for cleaning and defatting. After being dipped in Cresyl violet, the slides were taken through the final alcohols and xylenes prior to coverslipping using permount. A modification of Gundersen's fractionator technique for obtaining accurate regional counts of neurons was completed in order to specify the exact borders and intensity of damage within this general region.

2.6. Approximate localization of damage

An initial examination of each brain was made at 5 anterior–posterior levels (Bregma +1.7 mm, +0.7 mm, −0.3 mm, −1.3 mm, and −2.3 mm) in order to identify the approximate location of lesions in the neostriatum, globus pallidus, ventral pallidum or substantia innominata. Neuron counts were made using a light microscope (Leitz Laborlux S), a videocamera and a computerized videoimage analysis system (JAVA, Jandel) in nine regions at each level. The videoimage analysis system was used to project an image of the slice upon a monitor and exactly delineate the borders of the particular regions within the striatopallidal system. This technique led to a high consistency in terms of the region shapes and positions between the different rat brain slices. To determine the neuronal density within striatopallidal regions, neuron cell counts were completed using a eyepiece reticule (10 mm by 10 mm divided into 1.0 mm squares) placed into a 10 × eyepiece. The light microscope analysis was completed using a 20 × objective (total magnification 200 ×). A neuron was defined for scoring purposes as an intact circular or ovoid shaped object with a light purple interior stain, at least 10 microns in diameter (one-fifth of a 50 micron length individual grid). Both medium (10–15 microns in diameter) and large (> 15 microns in diameter) neurons were counted. The nucleus was not required to be present in order for an object to be counted as a neuron.

2.7. Fractionator analysis of lesion boundaries

Identified lesions were analyzed in detail using our modification [19] of the fractionator technique [33]. Twenty-five rats were selected for the modified fractionator technique (8 controls, 17 hyperkinetic paw treaders). This procedure was conducted in 3 stages.

Stage 1. Normal reference maps. First, in order to obtain accurate normal baseline neuron averages, the normal neuronal density of 251 brain fractions from 7 AP levels was determined using a modification of Gundersen's fractionator technique [33] (see Fig. 1). Fractions ranged in cubic volume from 0.03 mm^3 to 0.375 mm^3. The smallest fractions were grouped around sites of injections. For a complete listing of the normal neuron density for all fractions ($n = 251$) of the striatopallidal system see Table 1.

For each fraction, an exhaustive neuronal count was made of a 250 × 250 × 30 micron 'core sample'. This was done by delineating a 250 square micron area of a 30 micron tissue section (randomly chosen within each fraction) at 400 × magnification using the videoimage analysis system. Neurons were counted throughout the total volume of the fraction by counting all neurons at each plane of focus. Neuronal density varied from fraction to fraction in normal animals from 12 neurons in fractions from the globus pallidus to 154 neurons in fractions from the ventrolateral striatum. However, neuronal density varied by less than 25% between different rats for any given fraction. This consistency between animals in neuronal density for a given fraction allowed us to set a criterion for the detection of lesions.

Stage 2a. Identifying lesions by the fractionator method. 'Moderate neuron loss' was judged to exist if a fraction had less than 50% of the normal mean number of neurons for that fraction. Since normal brains vary by less than 25%, a neuronal density decrease below 50% falls well beyond the normal range. Areas that had greater than 50% neuron loss but less than 80% neuron loss were labeled as the shell of the lesion. 'Severe neuron loss' existed if the fraction had lost at least 80% of its neurons compared to the normal mean. Fractions which had the most severe neuron loss were labeled as the core of the lesion.

Stage 2b. Lesion border analysis. Once the center of the lesion had been located, 8 radial arms were drawn using the video image analysis system (JAVA System, Jandel, Corp.) emanating from this center along the major compass points (0°, 45°, 90°, 135° etc., Fig. 1 lower left). Core sample counts were taken along each line at 250 micron steps at 400 × magnification (see above) until the neuron density rose above 50% of the normal level for that fraction. Lesions borders were mapped onto stereotaxic atlas pictures of the representative brain slices [63] and were traced into the computer using a digitizing tablet and a 3-D reconstruction program (PC3D, Jandel). The digitized slices were converted into a 3-D representation for lesion viewing using a lateral, ventral, and a dorsal 3-D view.

Stage 3. Subtraction of noncrucial sites of damage. A composite map of shared damage was made for each syndrome group. This was done by first adding the mapped lesions of each rat that showed a particular syndrome together. Then any subregion of damage that was not shared unanimously by all brains within the group was subtracted away in order to eliminate areas of damage that were not necessary to produce the syndrome. The remaining composite lesion identified the common site that was damaged in every rat which displayed either hyperkinetic treading plus aversion or hyperkinetic treading alone.

2.8. GFAP-immunoreactivity

In order to confirm lesion placement by an independent measure, a second technique was employed that mapped regions of gliosis based on glial fibrillary acidic protein immunoreactivity (GFAP-IR). Glial fibrillary acidic protein immunoreactivity preferentially stains reactive astrocytes. Free-floating 30 micron sections were rinsed in three consecutive washes (PBS with 1% bovine serum albumin and 0.03% triton ×100). The slices were transferred to 1.5 ml centrifuge tubes containing rabbit anti-GFAP (primary, diluted 1:500, Dako Corp.) and placed on a roto-torque to turn slowly for 20–24 h at 5°C. The slices were rinsed in three consecutive PBS-BSA (0.03% triton ×100) washes and placed into 1.5 ml centrifuge tubes containing peroxidase conjugated to goat anti-rabbit IgG (secondary, diluted 1:100, Dako Corp.) and rotated for 1 h at room temperature. The slices were taken through a final set of rinses (PBS-BSA + 0.03% triton ×100) followed by an 8 min bath in freshly prepared 3,3-diaminobenzidine tetrahydrochloride on a shaker. The slices were then rinsed in distilled water, mounted directly onto a slide (gel-coated 2×), defatted and coverslipped using permount.

Areas of lesion-induced gliosis are much darker than the surrounding area after GFAP immunoreactive staining. Using a computerized densitometry video analysis based on pixel darkness (shades of grey from light grey to black) the reactive gliosis was calibrated, and a criterion of the darkest 20% of the pixels and the darkest 10% of the pixels was set and used to identify gliosis shell and core regions respectively. The gliosis shell and core were traced on to pictures of brain slices [63]. The lateral ventricle and corpus callosum were used as landmarks. Maps of lesions constructed by gliosis analysis were compared to maps constructed by neuronal density analysis.

2.9. Statistics

Group averages for taste reactions and paw treading duration were compared using the Mann–Whitney *U*-nonparametric test. Mean neuron counts for each striatopallidal region were compared across groups by a two factor (region × treatment group) analysis of variance (ANOVA). Differences across regions within lesion groups were analyzed by the a posteriori Newman–Keuls test. Means of regional neuron counts between lesion groups were also compared using the Newman–Keuls test.

3. Results

3.1. Behavioral results

Nine of eleven rats that received bilateral neurotoxin injections into the GP displayed hyperkinetic treading to quinine alone (T Syndrome). The average treading duration in this group was 14 s per quinine taste trial (compared to 0 s for controls, Mann–Whitney test, $P < 0.05$, see Fig. 2). No rat in this group showed treading to sucrose taste infusions. Taste reactions (besides exaggerated treading to quinine) did not differ from controls, and these rats ate and drank normal amounts.

Eight of twelve rats that received bilateral neurotoxin injections into the ventral pallidum/substantia innominata showed hyperkinetic treading to both quinine and sucrose solutions. These same rats (A + T Syndrome) also had a significant increase in the number of aversive responses [gapes, head-shakes, forelimb-flails and chin-rubbing] to sucrose (1 M) compared to sham-injected control rats, which showed

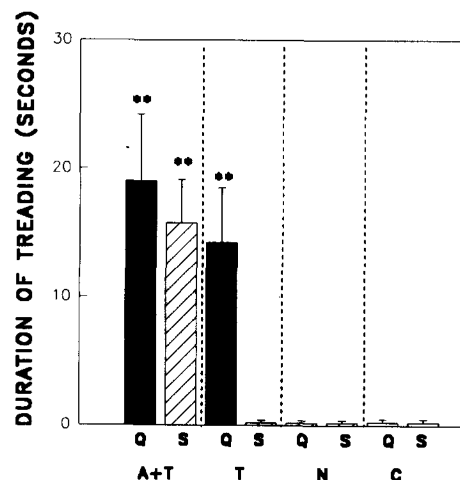

Fig. 2. Duration of hyperkinetic treading to either quinine (Q) or sucrose (S) oral infusions by: (1) rats that show the combined syndrome of exaggerated treading plus enhanced aversion (A+T Group); (2) rats that showed only the exaggerated treading syndrome without enhanced aversive taste reactivity (T Group); 3) rats with lesions that did not show either treading or enhanced aversion (N); and (4) sham-injected controls (C). ** and * denote significance at the $P < 0.001$ and $P < 0.05$ levels, respectively.

none. The average treading duration for this group was 19 s for quinine and 16 s for sucrose (Mann–Whitney test, $P < 0.01$ compared to 0 s for controls, see Fig. 2). The number of aversive responses to sucrose was increased to an average of 13.5 compared to 0 for controls (Mann–Whitney test, $P < 0.001$). Rats that showed enhanced aversion plus hyperkinetic treading (A + T Syndrome) were also aphagic and adipsic following surgery. The average length of aphagia and adipsia was 5 days (range, 2–7 days for these rats). The average weight loss was 64 g. General motor activity appeared suppressed and some rats were severely aki-

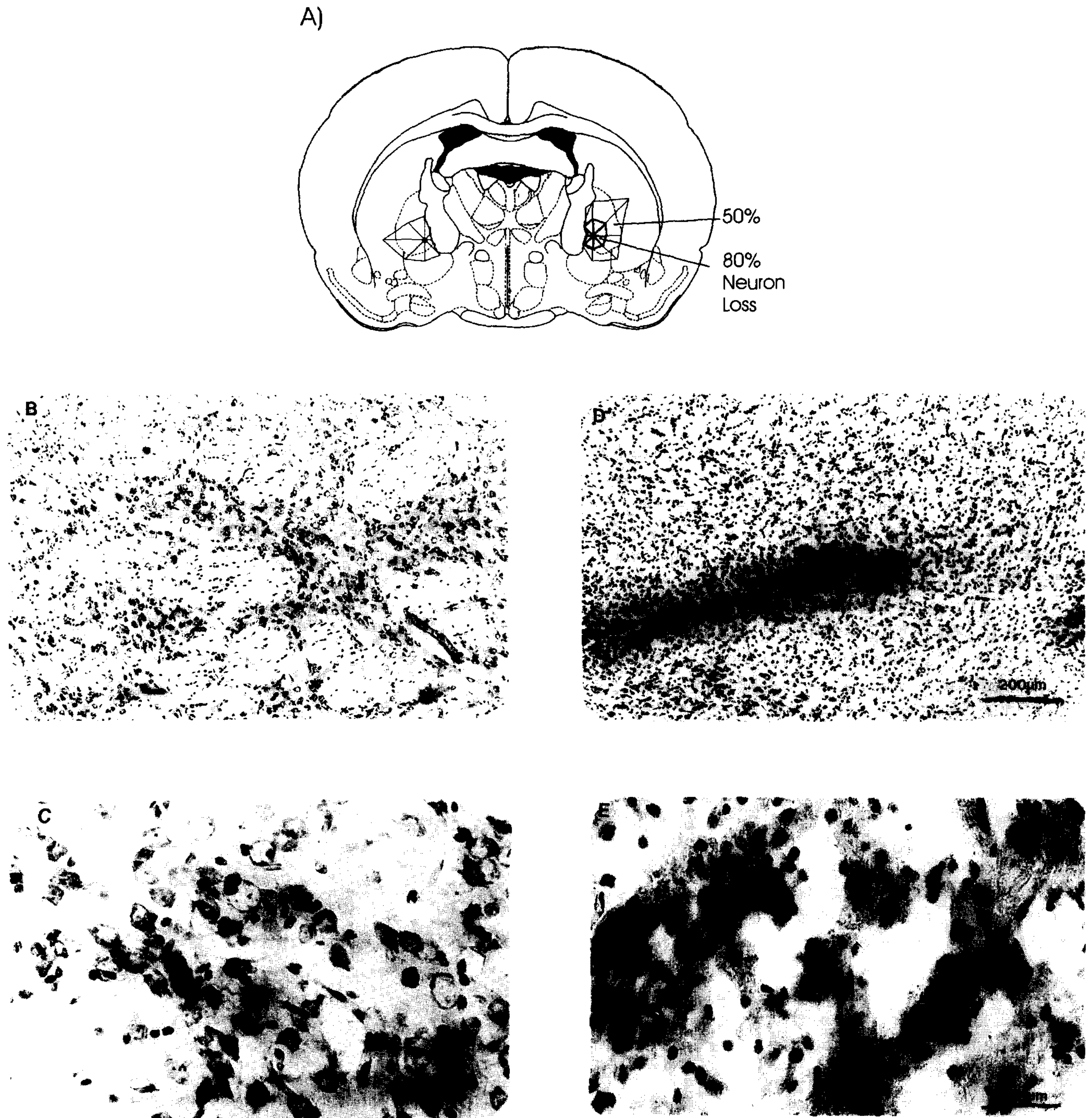

Fig. 3. T Group lesion. Representative lesion mapped to show the result of the fractionator and boundary analysis techniques (A), photomicrograph of the globus pallidus from a control rat (10 × magnification, B), and high magnification photomicrograph of the globus pallidus from a control rat (40 ×, C), photomicrograph of lesion from a T Group rat (10 ×, D) and a high magnification photomicrograph of the lesion core from a T Group rat (40 ×, E).

netic with postural deviations such as chronic limb extension.

Rats that received neurotoxin injections into either the ventral striatum or into any quadrant of the neostriatum did not show hyperkinetic treading or changes in taste reactivity (N Group).

3.2. Globus pallidus lesions: production of treading alone (T syndrome)

Lesion histology was completed 4–14 days post-surgery for the T-Group rats. In an analysis to detect shrinkage, measurements for striatal or pallidal width and length were similar for the T-Group rats and controls. This finding suggests that there was minimal shrinkage due to excitotoxic injury. Reactive astrogliosis was prominent in the lesion area and most likely filled in the regions of neuronal loss. For the T-Group rats with neurotoxin injections aimed at the GP that displayed hyperkinetic treading to quinine alone, significant bilateral damage compared to sham-injected controls was noted in only two regions: the globus pallidus and the ventrolateral neostriatum (Three-way ANOVA (Group × Region × AP level), $F(104, 712) = 4.806$, $P < 0.001$; globus pallidus and ventrolateral neostriatum regions were identified by post-hoc comparisons with control group, $P < 0.05$, Newman–Keuls). Both regions had bilateral damage located at Bregma -0.3 mm and -1.3 mm (globus pallidus $\geq 67\%$ bilateral damage; ventrolateral neostriatum $\geq 40\%$ neuron loss). No other regions distal from the injection site had bilateral damage.

In the more detailed fractionator analysis, a rat-by-rat examination was completed for neuron loss within the globus pallidus and ventrolateral neostriatum for the rats that showed the hyperkinetic treading to quinine alone. Each rat in this group had damage in a subregion of the globus pallidus (Fractions numbers 208 and 207; Fig. 3). The boundary analysis using neuron counts along the 8 radial arms emanating away from the center of the lesion for each rat, showed that each rat had damage in the ventromedial corner of the GP, immediately lateral to the internal capsule and dorsal to the VP/SI region (minimum damage = 60% bilateral neuron loss for each rat, see Fig. 4). The stereotaxic center for this crucial site, determined by subtraction analysis, was located at 1.3 mm posterior from bregma (at this AP level each rat had the largest lesion area). The lateral diameter of the common site was 0.8 mm, the dorsoventral diameter was 1.0 mm and the AP diameter was 1.0 mm.

Surrounding subregions of the GP were damaged in only some rats that showed the hyperkinetic treading to quinine. Eight out of 9 rats had > 50% damage to the overlying dorsal GP and 6 out of 9 rats had > 50% neuron loss in the lateral GP. Outside of the GP, the

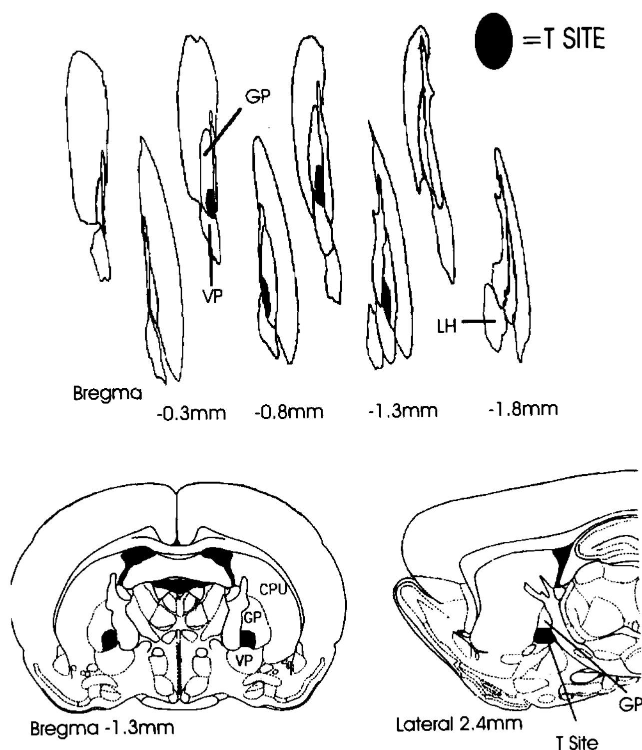

Fig. 4. Crucial site for the T syndrome as mapped by our modified fractionator procedure. The same views are shown as used in Fig. 6. Solid region denotes site where all rats had at least 60% neuron depletion.

ventrolateral neostriatum was bilaterally damaged in 2 out of the 9 rats. Ventral to the GP, the VP/SI or LH was bilaterally damaged in only 1 out of 9 rats. GFAP-IR had the darkest pattern of staining over the ventromedial GP for each of the rats showing hyperkinetic treading to quinine alone. This pattern of results supports the idea that damage to the ventromedial GP is sufficient to produce the hyperkinetic treading syndrome (T syndrome).

Significant GP damage was not seen in the non-symptomatic rats ($n = 2$) that received injections aimed at the GP. Unilateral damage of the GP was noted in both rats.

3.3. Ventral pallidum / substantia innominata lesions: production of treading plus aversion

Lesion histology for the A + T Group rats was completed between 4 and 15 days post-lesion. In an analysis to detect shrinkage, measurements of striatal and pallidal lengths and widths were similar between A + T Group rats and sham injected controls. This finding suggests that the total volume of the striatopallidal region was not significantly different between these two groups and that accurate neuronal counts for both regional and fractionator analyses could be obtained. A + T Group rats had significantly more damage in the

VP/SI region at Bregma −1.3 mm compared to both control rats and T Group rats (81% average depletion, Three-way ANOVA (Group × Region × AP level) $F(104,712) = 4.806$, $P < 0.001$; ventral pallidal region identified by post-hoc comparisons with controls and with T Group rats, $P < .0.05$, Newman–Keuls). Compared to only sham-injected controls the A + T Group rats also had significant bilateral neuron loss in the GP (34% neuron depletion, $P < 0.05$, Newman–Keuls) and in the LH (59% neuronal depletion, $P < 0.05$, Newman–Keuls). No other distal regions had bilateral neuronal damage.

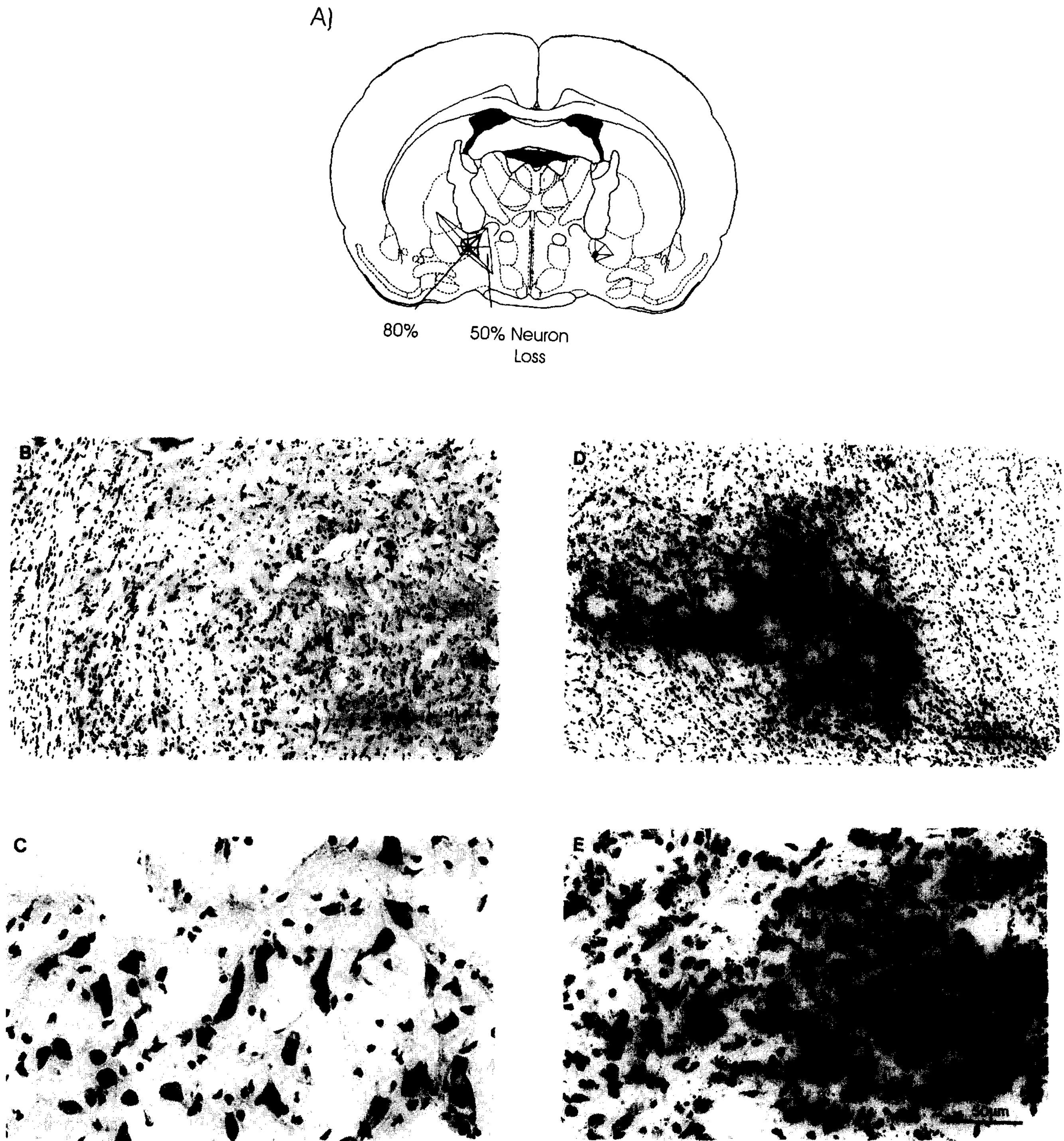

Fig. 5. A + T Group lesion. Representative lesion mapped to show the result of the fractionator and boundary analysis techniques (A), control photomicrographs of the ventromedial ventral pallidum/substantia innominata (B and C, same magnifications as in Fig. 3), low magnification (D) and high magnification (E) of a lesion from a rat in the A + T Group.

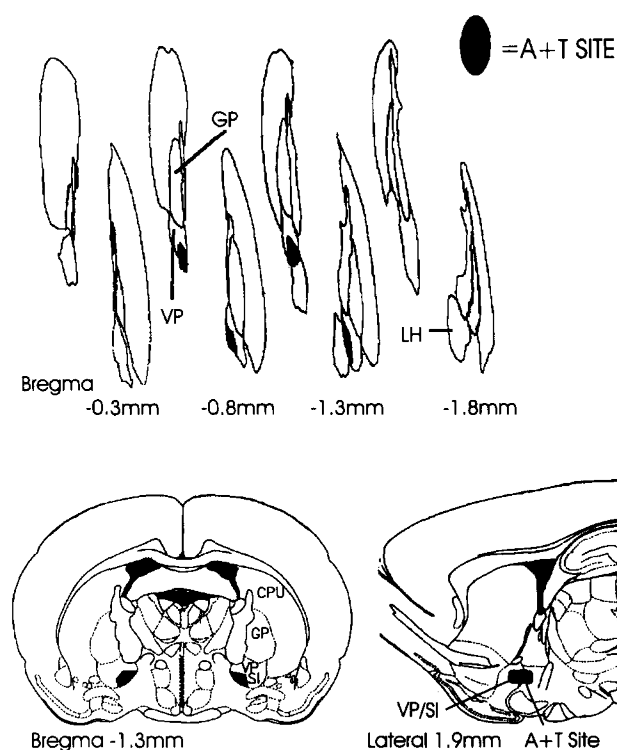

Fig. 6. Crucial site for the A+T syndrome. A 3-dimensional view (above) and coronal and sagittal 2-D views (below) of the 'necessary and sufficient' site of damage, as mapped by our modified fractionator procedure, that produces both hyperkinetic treading and an enhancement of aversive reactions to oral infusions. Solid region denotes site where all rats had at least 70% neuron depletion.

Results from the more detailed fractionator analysis of striatopallidal subregions showed that common damage was located only in a subregion of the VP/SI in the A + T Syndrome group (Fraction numbers 215 and 216; Fig. 5). The lesion boundary analysis using the 8 radial arms emanating from the lesion center for each rat showed that the common subregion within the VP/SI for the group was the ventromedial corner which is adjacent to, but outside of, the dorsolateral rim of the lateral hypothalamus and ventral and medial to the globus pallidus (see Fig. 6). Each rat had at least 70% neuronal depletion within this area of the VP/SI. The center for this common site was 1.3 mm posterior from bregma, 2.0 ± 0.5 mm lateral from bregma, and 7.3 ± 0.25 mm ventral to the skull as depicted in the stereotaxic atlas of Paxinos and Watson, 1986. The lateral diameter of the shared lesion site was 1.0 mm, the dorsoventral diameter was 0.5 mm and the AP diameter was 1.0 mm.

Subregions outside of the ventromedial VP/SI were damaged in some but not all rats that showed both enhanced aversion plus hyperkinetic treading (A + T Syndrome). Six out of 8 rats had > 50% neuronal depletion bilaterally in the adjacent dorsal ventral pallidum and 4 out of 8 rats had > 50% bilateral neuron loss in the lateral VP/SI. Outside of the VP/SI, the GP had > 50% bilateral neuron loss in 5 out of 8 rats, and ventrally, the LH had > 50% neuron loss in 4 out of 8 rats. No other structure was damaged in at least 4 of the rats. Dense GFAP-IR was measured over the ventromedial VP/SI in each rat that displayed the enhanced aversion plus hyperkinetic treading. These results support the conclusion that damage to the ventromedial VP/SI is sufficient to produce these behavioral changes.

Four rats that received injections aimed into the VP/SI did not show any behavioral changes and even though they had some bilateral neuron loss in the VP/SI compared to controls. These 'non-symptomatic' rats had much less severe damage compared to 'symptomatic' rats in the VP/SI (20–59% neuron loss in the non-symptomatic rats compared to a minimum of 70% bilateral neuron loss in each symptomatic rat). No non-symptomatic rat had bilateral damage exceeding 70%. This result indicates that a threshold of neuronal depletion is necessary for the production of the enhanced aversion and hyperkinetic treading syndrome.

3.4. Other lesion groups

Rats that received injections aimed at the ventral striatum had the most damage in this region (> 65% bilateral neuron loss). Rats that received neurotoxin injections into the different neostriatal quadrants had significant bilateral damage in those respective quadrants (dorsolateral neostriatal injection group had > 69% bilateral neuron loss in the dorsolateral neostriatum; dorsomedial neostriatal injection group had > 62% bilateral neuron loss in the dorsomedial neostriatum; ventrolateral neostriatal injection group had > 75% bilateral neuron loss within the ventrolateral neostriatum; and ventromedial neostriatum injection group had > 81% neuron loss in the ventromedial neostriatum). Any extra-striatal damage was seen unilaterally in the striatal lesion groups.

4. Discussion

Damage to two neuroanatomically separate sites was found to produce the two different behavioral syndromes of hyperkinetic treading originally differentiated by Berridge and Cromwell [11]. Lesions of the ventromedial third of the globus pallidus produced the sensory-triggered hyperkinetic treading syndrome alone (T Syndrome) but did not produce enhanced aversion to food (see Fig. 4). This T syndrome site is 1.0 mm by 0.8 mm by 1.0 mm in diameter and lies just lateral to the internal capsule and dorsal to the VP/SI (Fractions 207 and 208). Lesions of the ventromedial corner of the VP/SI produced hyperkinetic treading plus an

enhancement of aversive taste reactions to oral infusions (A + T Syndrome; for more discussion of 'aversion sites' see Cromwell and Berridge [19]). The crucial site appears for the A + T syndrome to be 1 mm by 0.5 mm by 1.0 mm in diameter and is located ventral and medial to the globus pallidus and immediately lateral to the border of the lateral hypothalamus (Fractions 215 and 216; Fig. 6).

The *site of damage* (rather than the diameter or the suprathreshold severity of a lesion) appears to be the primary determinant of which treading syndrome is produced. The two sites are approximately 0.5 mm apart. Lesions that produce treading plus enhanced aversion are ventral and medial to the lesions that produce the hyperkinetic treading alone, but they need not be larger or more severe than lesions that produce the treading syndrome alone. (so long as bilateral damage in the vmVP/SI, Fractions 215 and 216 in Fig. 3 exceeds > 60% neuron loss). Interestingly, ventral pallidal lesions that produce the combined syndrome need not damage the globus pallidus 'T Site'. However, lesions that do damage both sites always produce the combined syndrome of exaggerated treading to both sucrose and quinine, as well as aphagia and enhanced aversive taste reactions.

4.1. Ventromedial GP subregion: anatomical connectivity

The globus pallidus has been divided into ventromedial and dorsolateral sectors based upon differential neuroconnectivity of the sectors with other structures. The neostriatum or caudate nucleus and putamen (CPU) are the primary afferents to both sectors [38,61]. The subthalamic nucleus is the primary efferent [58,59]. The ventromedial GP, where lesions produce hyperkinetic treading to quinine, receives unique input from the ventromedial caudate nucleus and putamen (vmCPU) [60,61], which in turn receives its primary input from limbic structures: the hippocampus, amygdala, and cingulate cortex [24,51,61]. The ventromedial GP also receives direct input from the nucleus accumbens, which is part of the 'limbic' ventral striatopallidal system [43,53]. Injections of the GABA antagonist, picrotoxin, into the ventromedial GP abolish hyperlocomotion initiated by stimulation of the nucleus accumbens [43]. This direct influence of the ventral striatum upon the ventromedial globus pallidus has been argued to provide "a direct link between limbic and motor systems for the translation of motivation into action." (Jones and Mogenson, [43] p. 104.)

4.2. Ventromedial VP / SI subregion: anatomical connectivity

Both the A + T and T hyperkinetic syndromes have been shown to involve an interaction of sensorimotor and motivational (aversion) processes [11]. The T Syndrome is activated by naturally aversive stimuli, such as quinine, whereas the A + T Syndrome causes all tastes to be reacted to as aversive. Both the ventromedial VP/SI and the ventromedial GP have limbic neuroanatomical connections that make them distinctively different from dorsolateral subregions of the VP and GP [34–37,40,55,79]. Several researchers have suggested that the ventromedial subregions of the ventral pallidum/substantia innominata and globus pallidus have a special role in motivational and motor interaction [5,53,62,79].

The ventromedial VP/SI has a set of distinct connections, which for the most part are different from those of the dorsolateral VP/SI [29,30,39,78]. Specifically, the ventromedial VP/SI [28] receives input from the shell portion of the nucleus accumbens [41,78], the medial part of the bed nucleus of the stria terminalis (mBNST), the medial nucleus of the central amygdala (ceAM), the medial preoptic nucleus (MPO), the anterior hypothalamus (aHY), the medial area of the lateral hypothalamus (LH) and the ventromedial hypothalamus (VMH) [32]. This input to the ventromedial VP/SI from various limbic structures has reinforced the idea that ventral striatopallidal efferents participate in motivational processing [5,39,60].

The ventromedial VP/SI sends reciprocal connections back to the mBNST, ceAM, MPO, aHy, and LH [9,31]. Efferent projections also extend to the pedunculopontine nucleus (PPnT), the cortex, and the ventral tegmental area (VTA) [36,76,77]. Several researchers have proposed that the VP projections to either cortex or to the PPnT could allow access to sensorimotor processes [36,54,56]. The VP to PPnt connection has been shown to influence locomotion by experiments that used both electrophysiological and pharmacological techniques [52,54,55]. The ventral pallidal projection to the VTA region may also influence motor circuitry by modulating the dopamine neurons that project to the 'motor' dorsal striatum [30]. This ventral to dorsal striatopallidal connection through the midbrain could be important in integrating motivational and motor processes.

4.3. Neuropathological models and clinical perspectives

Recent models of hyperkinetic movements in humans postulates that disinhibition of the lateral globus pallidus (LGP) by degenerative striatal disease is the cause of the hyperkinetic movements of certain disorders [1,21,23,64,75]. Disinhibition of the LGP [3,4] is hypothesized by these models to be a result of a selective early degeneration of the co-localized GABA/ENK inhibitory striatopallidal projection. The

early loss of this projection [2] is consistent with post-mortem studies of brains of patients with Huntington's disease [65]. Since the LGP is thought to normally inhibit the subthalamic nucleus (STN), then, "the ultimate effect would be loss of the STN-derived excitatory drive to the MGP and SNr and disinhibition of the VA/VL/MD/CM-pf thalamocortical projection." (Albin et. al., [1] p. 370), resulting in hyper-excitation of the motor cortical areas and hyperkinesis.

Based upon these models, it could be predicted that pallidal destruction should lead to *hypokinesis*. The results of our lesion analysis, however, show that damage to the ventral pallidum/substantia innominata and globus pallidus can produce hyperkinetic treading movements triggered by oral sensory stimulation. Several factors could lead to this discrepancy between our results and the predictions made by these models of hyperkinetic disorders. The time progression and the selectivity [74] of neuropathology may be important in producing the differences between our results and the model's predictions. Human disorders usually are chronic diseases characterized by degeneration of a gradual nature. In contrast, our pathology was caused by an acute injection of excitotoxin that produced rapid onset of hyperkinetic symptoms. Acute injections of neurotoxic quinolinic acid appear to cause non-selective striatal neuron loss [12] (although this point is still controversial) [8]. More realistic techniques for mimicking Huntington's disease neuropathology have recently been devised, which use chronic injections (3–7 weeks) of smaller amounts of quinolinic acid [7,72]. For example, Bazzett and colleagues have found selective neuronal degeneration patterns within the dorsal striatopallidal system of rats after a chronic infusion of minute amounts of quinolinic acid (3.3 μmoles), not unlike that found in the post-mortem brain of a Huntington's disease patient [7]. A better test of the hyperkinetic disorder model [1,20,64,66,75], would be to combine chronic excitotoxin infusions into the striatum or globus pallidus with our behavioral test of sensory-triggered hyperkinetic treading.

In any case, our results indicate that hyperkinetic movements can be elicited from rats after pallidal damage. Could damage to the pallidal region be involved in human hyperkinetic disorders? Lange and colleagues found that the volume of the lateral globus pallidus was reduced in Huntington's chorea as much as the caudate nucleus and putamen (57% globus pallidal reduction compared to 56% striatal reduction) [47] This finding prompted Lange et.al. to note that, "In Huntington's chorea the pallidum was more severely affected than is commonly appreciated." (Lange et al., [47] p. 401).

Do hyperkinetic symptoms ever occur following lesions to the pallidal region without striatal damage in humans, as would be predicted from our observations in rats? Hyperkinetic symptoms have been reported in a number of human patients who have suffered acute damage to the region of the globus pallidus. For example, buccofacial dyskinesia and truncal choreic movements have been seen after specific bilateral damage to the GP in humans following carbon monoxide poisoning or hemorrhage involving this region bilaterally [48,69]. Specific hyperkinetic movements include face and jaw movements that resemble tics, and involuntary finger movements during 'mental counting'. The walking gait of such a patient was described as choreic in nature [48]. These motor symptoms are accompanied by drastic changes in motivation and cognition [48,69] which may be related to the motivational changes produced by our lesions [11]

5. Conclusions

In summary, this study mapped the location of two sites where neuron death produces two distinct sensory-triggered hyperkinetic syndromes. Damage to a site in the ventromedial globus pallidus (Fractions 207 and 208), exceeding a threshold of 60% bilateral neuron loss, produced the sensory-triggered treading syndrome but did not produce an increase in aversive taste reactions. The T syndrome site was approximately 0.8 mm × 1.0 mm × 1.0 mm in diameter. Damage to a ventromedial VP/SI site, exceeding 70% bilateral neuron loss, produced a combined syndrome of hyperkinetic treading and enhanced taste aversion to sensory triggers. The A + T syndrome site occupied the ventromedial corner of the ventral pallidum/substantia innominata (Fractions 215 and 216) and was ascertained to be approximately 1.0 mm × 0.5 mm × 1.0 mm in diameter (height, width, length). The determinant of which hyperkinetic syndrome was shown by an individual rat was found to be due to the placement of the lesion relative to these sites, rather than to the size or the suprathreshold severity of the excitotoxin lesions.

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