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The Vermicelli Handling Test: A Simple Quantitative Measure of Dexterous Forepaw Function in Rats

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Abstract

Loss of function in the hands occurs with many brain disorders, but there are few measures of skillful forepaw use in rats available to model these impairments that are both sensitive and simple to administer. Whishaw and Coles (1996) previously described the dexterous manner in which rats manipulate food items with their paws, including thin pieces of pasta. We set out to develop a measure of this food handling behavior that would be quantitative, easy to administer, sensitive to the effects of damage to sensory and motor systems of the CNS and useful for identifying the side of lateralized impairments. When rats handle 7 cm lengths of vermicelli, they manipulate the pasta by repeatedly adjusting the forepaw hold on the pasta piece. As operationally defined, these adjustments can be easily identified and counted by an experimenter without specialized equipment. After unilateral sensorimotor cortex (SMC) lesions, transient middle cerebral artery occlusion (MCAO) and striatal dopamine depleting (6-hydroxydopamine, 6-OHDA) lesions in adult rats, there were enduring reductions in adjustments made with the contralateral forepaw. Additional pasta handling characteristics distinguished between the lesion types. MCAO and 6-OHDA lesions increased the frequency of several identified atypical handling patterns. Severe dopamine depletion increased eating time and adjustments made with the ipsilateral forepaw. However, contralateral forepaw adjustment number most sensitively detected enduring impairments across lesion types. Because of its ease of administration and sensitivity to lateralized impairments in skilled forepaw use, this measure may be useful in rat models of upper extremity impairment.

Keywords

limb use; motor skills; manual dexterity; upper extremity impairment; stroke; brain injury; Parkinson's disease; reaching

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1. Introduction

Manual dexterity is central to daily activities and it is commonly disrupted by nervous system damage, often with permanent effects. Rodents use their forepaws in dexterous ways that are in some capacities homologous to humans (Iwaniuk and Whishaw, 2000; Cenci et al., 2002), and there is a growing appreciation that forepaw movements provide a useful model for probing aspects of hand function and dysfunction. The most well developed formal tests of forelimb function in rodents are the skilled reaching tests (a.k.a. reach-to-grasp tests), in which rats are placed in an enclosure and reach with the forepaw past some obstacle to grasp and retrieve food pieces (Peterson, 1934). This includes single pellet retrieval tasks (Castro, 1972; Whishaw et al., 1986), tray reaching tasks (Brácha et al., 1990; Whishaw and Pellis, 1990), the pasta matrix reaching task (Ballermann et al., 2001) and the Montoya staircase task (Montoya et al., 1991; reviewed in Nichols et al., 2005). These reaching tests are invaluable for their sensitivity to forelimb impairments resulting from CNS damage and for investigations of the neurobiology of motor skill learning (Greenough et al., 1985; Conner et al., 2003; Kleim et al., 2003) and neurorehabilitation after brain damage (Biernaskie and Corbett, 2001; DeBow et al., 2003; Kleim & Jones, in press; Maldonado et al., in press). However, they tend to be labor intensive to administer, which may sometimes preclude their use as one of a large battery of tests. They require specialized apparatus, shaping and, when used to assay lesion-induced impairments, may require extensive pre-operative training. The use of scheduled feeding and/or food restriction to motivate performance may also complicate their use in studies of dietary manipulations. It would be useful to have a quantitative measure of changes in skilled forepaw function that is easier and simpler to administer.

It has long been known that CNS damage can influence the way rodents grasp and handle food (Peterson, 1951; Castro, 1972; Kalil and Schneider, 1975). Whishaw and colleagues have previously described in detail how rats eat food pieces of various shapes and textures, including nuts, seeds, cereal, crickets and various types of pasta (Whishaw et al., 1992; Ivanco et al., 1996; Whishaw and Coles, 1996; Whishaw et al., 1998). They have found that rats skillfully manipulate the food with movements of the forepaws, including fine digit movements. The handling is often asymmetrical, e.g., with one paw performing more support functions as the other moves the piece. For example, when eating long thin pieces of dried pasta, rats were found to use one paw to hold the piece in a whole paw grasp and the other paw to guide the piece into the mouth, often with the digit tips. As the piece became shorter, the paws moved together into a symmetrical holding pattern. After ablation of the sensorimotor cortex, the contralateral paw was used less to hold the piece and, when it did, its movements were awkward and abnormal (Whishaw and Coles, 1996). Unilateral 6-OHDA lesions also resulted in atypical eating patterns and changes in movements used to manipulate the pasta, including increased movements made with the forepaw ipsilateral to the lesion (Whishaw et al., 1997).

Here, we capitalize upon and extend this prior work by Whishaw and others with the goal of developing a simple quantitative test of pasta handling in rats that would be highly sensitive to lateralized impairments in forepaw function. Forepaw adjustments were operationally defined as discrete instances of forepaw release followed by re-grasp of the pasta piece or reformation of the paw-hold using digit movements (extension-flexion and/or abduction-adduction). This definition includes a subset of what was previously operationally defined as “paw movements” by Whishaw et al. (1997). It was chosen as a variable of interest based on extensive pilot work suggesting that it was sensitive to the effects of even small cortical lesions. We also measured pasta handling strategies that were defined as sporadic and/or atypical based on their infrequency in intact rats. We then tested these measures in different lesion models to assay their sensitivity to CNS sensorimotor system damage.

2. Materials and Methods

2.1. Subjects

A total of 120 adult male rats were used. The Sprague-Dawley strain was used for the 6OHDA study and the Long-Evans Hooded strain was used for all other studies. Data from animals with sensorimotor cortex (SMC) lesions were collected from rats being used in other studies, as described below. Rats were housed in pairs or triplets in clear polycarbonate cages with wood shavings, received water *ad libitum* and were maintained on a 12:12 hour light:dark cycle. Ages were 3.5 months (6OHDA study, N = 25), 4 months (electrolytic SMC lesion study, N = 8), 5 - 5.5 months (MCAO study, N = 12) and 6 months (ischemic SMC lesion study, N = 29) at the time of surgery. Surgically naive rats used to characterize vermicelli handling behavior were 4 months old (N=46). All animal use was in accordance with protocols approved by the University of Texas at Austin Animal Care and Use Committee.

2.2. Vermicelli Handling Test

2.2.1. Stimuli—Rats were given 7 cm lengths of uncooked vermicelli (1.5 mm diameter; 0.15 g/piece; Skinner brand, distributed by New World Pasta Co., Harrisburg, PA). For most tests, pieces were marked with an ultrafine tip marker at 1.75 cm intervals to facilitate visualization of the movement of the strand. A single source of Italian style vermicelli was used because of concerns that variations in texture and diameter may influence handling patterns and/or time to eat. Ingredients (provided by the manufacturer) were: semolina, durum flour, niacin, ferrous sulfate, thiamin, mononitrate, riboflavin, folic acid (calories per gram: fat 9, carbohydrate 4, protein 4). Prior to the onset of behavioral testing, all rats were given vermicelli pieces in their homecages to be made accustomed to pasta handling. Rats were not exposed to pieces longer than 7 cm because, in early work, it was discovered that rats given longer strands often adopted the strategy of breaking them into smaller pieces prior to eating, which confounds the test.

2.2.2. Vermicelli Handling Trials—A test consisted of 4-5 trials with pasta pieces given one at a time per each trial (Fig. 1). Rats were isolated from cagemates for the testing period and were tested either in their homecages or in similar cages with clear, unmarred, cage walls. All rats were accustomed to pasta eating in the presence of the experimenter prior to testing and were given 2-3 pieces as warm up trials prior to each test. Most rats behaved as if the pasta was highly palatable; thus the animals were not typically food restricted. However, removal of rat chow a few hours before or overnight was occasionally used to instigate eating in the presence of the experimenter. With experimenters practiced in this task, the trials could be performed in real time. Alternatively, trials were videotaped and replayed in slow motion. Data were collected without knowledge of experimental condition and/or injury side.

Data were collected with the rat facing the experimenter such that the digits and joints of the metacarpals and phalanges (knuckles) of both forepaws could be seen. By dropping the piece into a conveniently viewed part of the cage, rats could typically be encouraged to eat the piece with the paws facing the experimenter and/or video camera. Trials were ignored if the rat moved away or to the side, such that the digits and knuckles could not be seen well. Excess shavings were removed if they obscured the view of the paws. Two methods were used to count paw adjustments during pasta eating. Experimenters either used tally counters in each hand to record adjustments with the left and right paw separately, or, left and right paw adjustments were counted in alternating trials. In video recorded tests, many rats initially turned away from the camera but could be habituated to its presence in a few minutes. For particularly camera shy rats, a black plastic ring (the filter basket of a large coffee percolator) approximating the size of the camera lens hood was attached to their cage for a day or more, which prevented later distraction from the presence of the camera. Using these approaches, all rats could be made to eat the pasta pieces facing the lens of the camera. The camera used was a Canon XL1 zoomed

to magnify the paws and with a shutter speed of 1/420 s. The lens was positioned at approximately cage floor level angled slightly upward.

Note that the testing in these studies was performed in the home cage or similar cage merely for speed and convenience. We have found that this test can also be performed in other enclosures after rats are habituated to eating in the novel environment. One rat treated with 6-OHDA ate in an extreme abnormal posture that prevented sight of paw adjustments. Data from this animal were excluded. Although not done in this study, this might be dealt with by filming in a shavings-free chamber with the camera angled to view the rats from below, as was done by Whishaw and Coles (1996), provided that digits and knuckles can be clearly viewed.

2.2.3. Typical Pasta Holding Patterns—As previously described by Whishaw et al. (1996), when eating long thin pieces of pasta, intact rats most often hold the piece in both paws and move it into the mouth using a coordinated asymmetrical holding pattern (Fig. 1B). One paw, which we refer to as the “grasp” paw, holds the pasta in a whole-paw grasp, typically positioned lower (away from the mouth) on the piece at the start of eating. The “guide” paw is held closer to the mouth at the beginning of eating and appears to be used more to guide the piece into the mouth. Frequently, the guide paw holds the pasta between one or two digits and the thumb nub. When this asymmetrical eating pattern is used, the paws move together as the pasta strand becomes shorter, such that one paw is directly above the other (Fig. 1C) and, towards the end of the piece, paws are usually repositioned in apposition to one another, with digits interposed (Fig. 1D). The limb used (left versus right) in the grasp and guide roles, as determined at the onset of eating, was recorded for each trial.

Note that the terms “grasp” and “guide” indicate relative roles. Each paw is involved in both grasping and guiding the pasta piece during a typical trial. Both “grasp” and “guide” paws make adjustments. Frequently, rats were seen to make more adjustments with either the grasp or guide paw early in eating. As the paws moved together, adjustments with the paws often alternated or were performed together. However, rats appear to have a flexible repertoire of pasta handling behaviors and many variations in this pattern were seen between and within rats, as reported below.

2.2.4. Forepaw Adjustments—The primary quantitative variable recorded was the number of adjustments made with each forepaw per each pasta piece. These adjustments are obvious even to an inexperienced rater (Fig. 2). An adjustment was defined as any visible release-regrasp of the pasta piece or reformation of the paw-hold on the pasta piece using extension-flexion and/or abduction-adduction of the digits. Only adjustments made after eating had commenced were counted. Only adjustments in the paws' contact with the pasta piece, or with the other paw as it held the pasta piece, were counted. (Adjustments in the contact with the other paw were counted because, in slow motion in video play back, they were frequently revealed to also be adjustments in the contact with the pasta piece.) Not counted were instances of sliding the paw up or down on the pasta piece using wrist, elbow, shoulder and/or mouth movements, without movements of the digits. Also not counted were grasping or other movements made next to the pasta piece (i.e., movements initiated without physical contact with the pasta or other paw). This operational definition includes a subset of paw movements defined by Whishaw et al. (1997) as “...any grasping movement beside or on the pasta and any postural adjustments...” (pg. 169). In addition to the total number of adjustments made, the asymmetry in adjustments after the lesions was also calculated as % (ipsi-to-lesion forepaw adjustments/total adjustments).

The time to eat, beginning when the pasta piece was grasped and placed in the mouth and ending when the piece was released by the paws and disappeared into the mouth, was also

recorded. The sounds that rats make when eating pasta are audible and can be used to determine the onset of eating.

2.2.5. Atypical and Sporadic Behaviors—The most common pasta handling pattern in intact animals was the asymmetrical paw hold, but eating behaviors varied between rats and within rats between trials. Observations of atypical or sporadic pasta handling behaviors were recorded, as shown in Figure 3. Most of these behaviors are similar to those previously described by Whishaw and colleagues (Whishaw & Coles, 1996; Whishaw et al., 1997; 1998). Preliminary data in intact rats indicated that these behaviors, listed below, are not seen in the majority of trials and that they sometimes increase in frequency after injury. They were therefore chosen for quantification.

The atypical behaviors are: (1) *Paws together when long*, defined as holding the paws in symmetrical apposition, without a clearly defined grasp versus guide paw, when the pasta piece was 3.5 cm or greater in length; (2) *Guide and grasp switch*, the roles of the guide limb and grasp limb are switched during eating; (3) *Failure to contact*, the paw does not contact the pasta during eating, excluding instances of forepaw adjustments; (4) *Drop*, the pasta piece is dropped after eating is initiated; (5) *Paws apart when short*, the paws fail to move into a symmetrical position before the piece is completely eaten; (6) *Mouth pulling*, the mouth is used to pull the pasta piece through the paws; (7) *Hunched posture*, the rat hunches over the pasta piece and moves the mouth down as the piece becomes smaller, sometimes also supporting the pasta piece on the floor. For observations in 6-OHDA rats, three additional atypical behavioral categories were added based on previous descriptions (Whishaw et al., 1997) and preliminary observations: (8) *Iron grip*, one limb is held in an unmoving, rigid-appearing whole-paw grasp of the piece for most of eating; (9) *Guide around grasp*, the guide limb is repositioned repeatedly and rapidly above and below the grasp limb in an apparent compensatory manner to manipulate the pasta piece; (10) *Angling with head tilt*, the pasta piece is held in the mouth at an extreme angle as the head is tilted. (After 6-OHDA lesions, the pasta is angled into the unimpaired side of the mouth.) Angling with head tilt could obscure the identification of hunched posture and, therefore, the presence of this behavior was considered to preclude category 7 for the 6OHDA study. Categories 1, 5, and 7-10 were recorded as the number of trials in which they occurred (i.e., a maximum of 1 per trial). For the other categories, each occurrence was recorded. These data are reported as the number of occurrences per 4 trials.

2.3. Lesion Conditions

Animals were anesthetized, secured in a stereotaxic frame and received one of the following unilateral lesions. All rats received buprenorphine (0.05mg/kg) for peri-operative analgesia. MCAO and cortical lesions were performed using a cocktail of ketamine (100 mg/kg) and xylazine (10-12 mg/kg) for anesthesia. With the exception of the MCAO study, lesion side was not chosen with regard to any preexisting asymmetry in pasta handling adjustments. MCAOs were made contralateral to the forepaw making more adjustments in preoperative tests, as described below. There were similar numbers of left and right lesions for each lesion type (n 's ≤ 2 difference in left and right lesions per study).

Ischemic Sensorimotor Cortex Lesions—Lesions were made using topical application of the vasoconstrictor, endothelin-1, as described previously (Adkins et al., 2004). Briefly, the skull was removed between 1.0 mm posterior and 2.0 mm anterior to Bregma and between 3 to 4.5 mm lateral to midline. The dura was lifted and 2.5 μ l of 80 μ M endothelin-1 was dropped onto the exposed cortex and the animal was left undisturbed for 10 min. Controls received all procedures up to, but not including, the craniectomy. These rats were used in an ongoing study involving forelimb constraint procedures. Rats serving as no-constraint controls were included in the present analysis. These rats were placed in light-weight rat jackets (Lomir, Quebec,

Canada) which permit unrestricted movements for the first 12 days after surgery. The vermicelli handling test was administered pre-operatively and at several time points between 8 and 45 days post-operatively.

Electrolytic Sensorimotor Cortex Lesions—Lesions were made using an uninsulated platinum wire electrode (0.25 mm diameter) as described previously (Voorhies and Jones, 2002). Skull and dura were removed between 2.5 mm anterior and 1.5 mm posterior to Bregma and 1.5 to 4.5 mm lateral to midline. The electrode was lowered to 1.5 mm below dura and moved in 5 equally spaced traverses through the exposed cortex while 1 μ A anodal current was passed for a total of 2.5 min. These animals were tested preoperatively and on day 4 or 5 post-lesion. These rats were single lesion controls in a study of the effects of bilateral SMC lesions (Maldonado et al., 2006).

Middle Cerebral Artery Occlusion—The endothelin-1 method described by Windle et al. (2006) was used to produce MCAOs. A hole was drilled in the skull (0.9 mm anterior to bregma and 5.2 mm lateral to midline) and a microsyringe needle was lowered to 8.6 mm below dura. Endothelin-1 (4 μ l, 80 μ M, 0.2 μ g/ μ l, Peninsula Laboratories, Inc.) was infused at a rate of 0.01 μ l/sec. Following the infusion, the cannula was left in place for 5 min prior to its slow withdrawal. Controls received infusions of sterile saline. Pasta handling tests were performed pre-operatively and at time points between 2 and 28 days post-operatively. In this set of animals, lesions or sham-operations were intentionally made contralateral to the limb that the animals used most for adjustments during pre-operative testing.

Striatal Dopamine Depleting Lesions—Rats were pretreated with 0.1 mg/kg atropine sulfate s.c. (to dry respiratory tract secretions) and 20 mg/kg desipramine i.p. (to protect norepinephrine neurons) 10 min prior to anesthesia. Anesthesia was induced with 40 mg/kg pentobarbital i.p. and maintained throughout surgery with booster injections of 80 mg/kg chloral hydrate i.p. as needed. A small hole was drilled through the skull at 1.5 mm lateral to midline and 4.3 mm posterior to bregma. A microsyringe needle was slowly lowered through the center of this hole to a point 8.0 mm ventral to the dural surface, to target nigrostriatal dopaminergic axons in the medial forebrain bundle. A solution of 8 μ g (free base weight) of 6-OHDA HBr (a catecholamine neurotoxin; Sigma) dissolved in 2 μ l of artificial cerebrospinal fluid (ACSF) containing 0.05% (w/v) ascorbic acid was infused at a rate of 0.2 μ l/min. Sham-operated controls received infusions of the vehicle (ACSF plus ascorbic acid). At the end of the 10 min infusion the needle was left in place for an additional 2 min before being slowly retracted. The skull hole was sealed with bone wax. These rats were tested approximately 6-7 weeks postoperatively.

2.4. Lesion Verification

SMC and MCAO lesions—Lesions were verified in histological coronal sections, as described previously (Allred and Jones, 2004). Briefly, animals were overdosed with sodium pentobarbital, were perfused intracardially with buffer and fixative solutions, brains were removed and 50 μ m coronal sections were produced using a vibratome (Leica VT1000S). Sections were stained with toluidine blue (a Nissl stain) and lesion volumes were indirectly estimated by the method of interhemispheric volume differences. NeuroLucida software (MicroBrightfield, Inc.) was used to measure areas from coronal sections, viewed at X 49 magnification. For cortical lesions, volume measures were focused on the cortex within the SMC region (between +2.7 to -0.5 mm relative to bregma, sections 600 μ m apart). This focused region of analysis sensitively estimates the volumes of these relatively small lesions. For MCAO, which created mixed cortical and subcortical infarcts spanning a large region of the cerebrum, whole cerebral hemispheres were traced in sections 900 μ m apart. All volumes were estimated as the Σ area $\cdot$ distance between section planes. Tissue loss was estimated as the %

contra volume, calculated as ((contra-ipsi volume)/contra volume). Representative lesions are shown in Figure 4.

6-OHDA lesions—Levels of dopamine depletion were assayed using high pressure liquid chromatography with electrochemical detection (HPLC-ED) for dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). These data were used to divide animals into 3 depletion conditions: severe, partial and failed+sham (Table 1). Rats were anesthetized with halothane and decapitated. Brains were removed and the dorsal halves of the left and right striata were each dissected out over wet ice. Striata were then blotted free of excess moisture and accurately weighed before being sonicated in 40 μ l of cold mobile phase (see below) per mg of tissue obtained. The sonicated suspension was then centrifuged at 13,500 RPM for 15 min at 4°C and the supernatant was removed for analysis. Ten μ l of each sample was injected onto a BDS Hypersil C18 100 $\times$ 3 mm column (held at 40°C, and preceded by a 10 mm C18 guard column). The mobile phase consisted of a 15 mM sodium acetate / 20 mM citric acid buffer (pH 3.7) containing 70 μ M disodium EDTA, 0.04% (w/v) sodium 1-heptanesulfonate, and 10% (v/v) methanol. This mobile phase was pumped through the system at 0.9 ml/min with an ESA Model 582 delivery pump. A guard cell set at +450 mV was placed between the pump and sample injector to reduce electroactive impurities in the mobile phase. Eluates from the column were analyzed by an ESA Model 5011A analytical cell coupled to a Coulochem II controller. The first, coulometric (screening) electrode was set at a potential of -100 mV and the second, amperometric (detecting) electrode was maintained at +400 mV. The heights and retention times of current peaks recorded from the detecting electrode (as calculated by integration with ESA's Chromatography Data System version 1.1 software) were compared against those produced by a series of standards (obtained from Sigma) of known concentration to ultimately determine the striatal content of dopamine and its metabolites, DOPAC and HVA, in terms of μ g of substance per mg of wet tissue weight. When samples fell below the limit of detection for a given compound, a value halfway between zero and the detection limit was recorded for that sample.

2.5. Statistical Analyses

Behavioral data from lesion studies were analyzed using analyses of variance (ANOVAs) for groups (lesion versus sham-control group) and/or days (pre- versus post-operative) with the SAS general linear models (GLM) procedure. For the ischemic SMC lesions, repeated measures ANOVAs for the effects of group, day and group by day were used. SAS univariate contrasts were used to analyze group differences for individual days. MCAO and electrolytic SMC lesion studies were analyzed using repeated measures ANOVAs for the effects of day of testing (pre- versus post-operative). MCAO rats were additionally compared with a small group of sham-operates. For the MCAO study, some pasta handling sessions were filmed and, on the day before or after, data were also collected in real time. After statistical analyses indicated no significant differences, these data were pooled for the purpose of evaluating MCAO effects, but are also considered separately, as reported below. For the 6-OHDA study, no significant differences were found in catecholamine contents of the sham-depleted and failed depletions and these groups were combined for further analyses. 6-OHDA effects were analyzed with SAS univariate contrasts to compare partial and severe depletion groups with normals. Distributions of atypical behaviors were analyzed using chi-square analysis. Data in intact animals were characterized descriptively. Bivariate correlational analyses were used to assess the relationship between specific variables. All descriptive statistics are reported as means $\pm$ SEM unless otherwise noted.

3. Results

3.1. Pasta Handling Behavior in Intact animals

To characterize normal pasta handling, pre-lesion data from the studies reported below and data from an additional 46 naive rats were analyzed together ($n = 95$). These intact rats made 15.57 ± 0.57 adjustments per pasta piece (left + right combined). Averaged over the population examined, there was no major asymmetry in the two forepaws in pasta adjustments. Rats made $51.9 \pm 1.7\%$ of their adjustments with the left paw. However, many individual rats did make more adjustments with one paw than with the other. If preferential forepaw use is defined as using one paw for $\geq 70\%$ of adjustments, then 29/95 rats had a preferred paw for pasta handling. Of these 29 rats, 55% preferred the left paw.

As previously mentioned, rats most often began to eat the pasta using an asymmetrical holding pattern, where one paw is used primarily for grasping and the other for guiding the piece (Fig. 1). All but one rat displayed this pattern at the onset of eating in at least some of the trials and, averaged over all rats, this pattern was used in $92 \pm 2\%$ of trials. In trials in which an asymmetrical grasping pattern was not used, rats held the pasta piece between forepaws symmetrically (Fig. 3A). Approximately half of the rats (51.6%) always used the left ($n=26$) or always used the right ($n=23$) paws in the grasp role. The remainder changed Grasp paws between trials (34.7%) or used a mixture of asymmetrical and symmetrical holding patterns in different trials (13.7%). If a limb preference is defined as rats using the same paw in the grasp role in $\geq 75\%$ of trials (3 out of 4 trials), then 76.8% of intact rats had preferred paws for the grasp and guide roles.

A paw used in the grasp role tended to make more adjustments (adjustments made by Grasp paw: 8.61 ± 0.46 , Guide paw: 6.90 ± 0.31 , Symmetrically positioned paw: 5.73 ± 0.66). However, having a preferred-for-adjustment limb did not strongly predispose animals to use this limb in either the grasp or guide role. Considering only rats that made $\geq 70\%$ of adjustments with one limb, this limb was used in the role of the grasp limb in $59.2 \pm 7.3\%$ of trials.

Rats ate the pasta piece in 23.71 ± 0.98 sec making 0.75 ± 0.04 adjustments per sec. The distribution of pasta eating time was skewed towards the upper range (skewness = 1.35) and had a high kurtosis (3.11). However, if two rats that averaged more than 58 sec/piece are treated as outliers, these data had a more normal distribution (skewness and kurtosis = 0.58 and 0.17, respectively). Skewness and kurtosis were between -1 to +1 for adjustment number, rate and asymmetry.

Of the atypical/sporadic behaviors quantified in intact rats (categories 1-7, Fig. 3A-G), by far the most common was the category of paws together when long, which was observed on average 1.61 ± 0.14 times per four trials. Of the remainder, the most to the least frequent observations were grasp and guide switch (0.23 times/4 trials), failure to contact (0.11), drop (0.07), abnormal paws together when short (0.04) and mouth pulling (0.01). Hunched posture was not observed in intact rats. Summed together, these behaviors occurred 2.07 ± 0.16 times per 4 trials.

3.2. Sensorimotor Cortex Lesion Effects

As shown in Figure 5, ischemic SMC lesions resulted in enduring reductions in adjustments made with the contralateral forepaw for pasta handling in comparison to sham-operated controls. In repeated measures ANOVA, there was a main effect of group ($F(1, 27) = 16.83$, $P < 0.0003$) and a significant group by day interaction ($F(4,108) = 3.15$, $P = 0.017$). In post-hoc analyses, lesion groups had significantly fewer contralateral adjustments compared with sham-operates on each day of testing with the exception of the pre-operative time point. In

contrast, there were no significant group or group by day interaction effects in adjustments made with the ipsilateral forelimb, in the time to eat, nor in the occurrence of atypical behaviors.

In contrast to the MCAO study described below, the side of the SMC lesions was *not* chosen with regard to endogenous asymmetries in adjustments. As shown in Figure 5B, when SMC lesion groups were subdivided based on endogenous pre-operative asymmetry, it became apparent that the cortical lesion effects were more sensitively detected in animals in which the impaired limb was used more pre-operatively for adjustments. Rats that made 60% or more of pre-operative adjustments with the ipsilateral forelimb were assigned to the Ipsi Bias subgroup and those making 55% or fewer adjustments with this limb were assigned to the Contra or No Bias subgroup. There was a significant post-lesion reduction in both subgroups of rats (Ipsi subgroup: $F(1,6) = 15.74$, $P = 0.007$; Contra/No Bias subgroup: $F(1, 10) = 39.28$, $P < 0.0001$). However, the magnitude of the reduction in the Contra/No Bias was significantly greater than that found in the rats with a pre-existing ipsilateral bias ($F(1,16) = 11.53$, $P = 0.004$).

There were no significant post-lesion change or group difference in the use of the forelimbs in the grasp versus guide roles compared with sham-operates. Ischemic rats used the to-be-impaired limb as a grasp limb in $39.89 \pm 9.09\%$ of trials pre-operatively and in $33.33 \pm 9.48\%$ of the trials on post-lesion Day 8.

Electrolytic SMC lesions ($n=8$) also resulted in a significant reduction in adjustments with the contralateral forepaw at the single post-lesion time point examined. The contralateral forelimb made 1.2 ± 0.3 adjustments per piece after the lesion compared to 4.8 ± 1.2 adjustments prior to the lesion (pre versus postlesion time point, $F(1,7) = 8.96$, $P = 0.02$). As with the ischemic lesions, there were no significant changes in the time to eat nor in the use of the forelimbs in grasp and guide roles. However, the number of adjustments made with the ipsilateral forelimb tended to be greater after the lesion ($F(1,7) = 5.31$, $P = 0.055$). Rats made 6.8 ± 1.3 ipsilateral adjustments prior to the lesions and 9.9 ± 1.9 after the lesions. Furthermore, the number of atypical behaviors was significantly increased from 2.38 ± 0.56 to 3.88 ± 0.69 observations per 4 trials ($F(1,7) = 7.00$, $p = 0.03$). The increase was found in 4 of 7 rats and was primarily due to an increase in failures to contact and abnormal paws apart when short (Figs. 3C and E).

3.3. Middle Cerebral Artery Occlusion Effects

As shown in Figure 6, MCAO resulted in a significant and prolonged reduction in adjustments made with the contralateral forelimb. In repeated measures ANOVA, there was a significant effect of day of testing in the MCAO rats ($F(6,49) = 3.63$, $P = 0.0047$). In post-hoc analyses, contra adjustment numbers were reduced at every post-operative time point compared to the pre-operative time point. Adjustments with the ipsilateral limb, time to eat and use of the paws in grasp versus guide roles were not significantly changed by MCAO. In sham-operates ($n=4$), there were no significant changes in any of the variables with time.

MCAO increased the frequency of atypical behaviors, but, compared with the preoperative time point, this increase reached significance only on day 2 after the lesions. MCAO was also significantly different compared with the sham-operates ($F(1,10) = 6.44$, $P = 0.03$). The atypical behavioral categories most increased relative to the preoperative time point were drops (1.9 ± 1.7 more observations/4 trials postoperatively than pre-operatively), grasp and guide switch (1.1 ± 0.7), failure to contact (0.7 ± 0.4) and hunched posture (0.5 ± 0.3). Summed across categories, atypical behavioral observations were increased in every rat after the MCAO, though there was no single atypical behavior that was increased in the majority of rats. Increased switching of grasp and guide roles was seen in 4 out of 8 rats. Increases in drops, failures to contact and hunched posture were each observed in 3 rats. Some rats with MCAO were also observed to make “phantom” adjustments, i.e., grasp-release movements without

contacting the pasta piece. (These were not included in the counts of atypical behaviors in the present study.)

3.4. Striatal Dopamine Depletion Effects

6-OHDA lesions influenced pasta handling in a manner that varied with the severity of the depletion, as shown in Figure 7. In both partial and severe depletion groups, there was a reduction in the number of adjustments made with the forepaw contralateral to the depletion compared with the combined group of sham and failed-depletion control rats ($F(1, 22) = 8.67$ and 15.15 , respectively, P 's < 0.01). In the severely depleted animals, there was also a major increase in the number of adjustments made with the ipsilateral forepaw compared with normal rats ($F(1, 22) = 38.58$, $P < 0.0001$). This increase was not found in rats with partial depletions. Furthermore, there were significant increases in the time to eat the pasta after severe ($F(1, 22) = 20.50$, $P = 0.0002$), but not partial, depletions (Fig. 7B).

Another characteristic of the dopamine-depleting lesions was an increase in the use of the *impaired* forelimb as the grasp paw and decreased use of the ipsilateral paw in this role, as defined at the onset of eating. The contralateral paw was used in the grasp role in 97 ± 3 % of trials in severely depleted rats, 86 ± 10 % of trials in partially depleted and 67 ± 12 % of trials in normals (P 's < 0.05). However, the quality of the grasp was highly abnormal in the severely depleted rats because it was maintained with little to no adjustments during eating (i.e., in an “iron grip”) while the other paw alternated position above, below and over the grasp paw (Fig. 3H-I). This was seen along with head tilt in 7 of 8 of the severely depleted animals, 1 of 5 of the partially depleted animals and none of the normal controls (Table 2). After this, the most frequent behaviors were grasp and guide switch (1.0 ± 0.5 times/4 trials in severe depletions, 1.8 ± 0.8 in partial depletions and 0.4 ± 0.2 in controls) and drops (0.8 ± 0.4 , 0.2 ± 0.2 and 0 ± 0 , respectively). (Note that angling with head tilt was considered to preclude category 7, “hunched posture”.) There were significant overall increases in the frequency of atypical behavioral observations, as shown in Figure 7C.

3.5. Relationship between Lesion Severity and Pasta Handling Variables

SMC Lesions—SMC lesions resulted in focal damage to the forelimb region of the sensorimotor cortex (Fig. 4A-B). In the ischemic hemisphere, the volume of the SMC region was 11.5 ± 0.9 mm³ smaller than the intact cortex, a loss of 13.6 ± 1.0 % of the SMC region (% [intact-lesion]/intact volume). The loss in individual rats ranged from 6.7 to 23%. Electrolytic lesions resulted in a loss of 11.4 ± 1.1 mm³, which was 13.6 ± 1.2 % of the intact SMC (range = 8.8 to 17.2 %). There tended to be a positive correlation between percent tissue loss and asymmetry in adjustments, but this failed to reach significance in either lesion type or when ischemic and electrolytic lesions were combined for the analysis ($r = 0.39$, $P = 0.054$).

MCAO—MCAO resulted in major tissue loss. The infarcted hemisphere had 84.3 ± 20.4 mm³ less volume than the intact hemisphere. The tissue loss was 19.7 ± 4.8 % of the intact cerebrum and there was considerable range (3.7 to 39.6%). There were significant correlations between tissue loss and asymmetry in adjustments ($r = 0.82$, $P = 0.0012$), as well as atypical observation number ($r = 0.68$, $P = 0.015$) as assayed at post-lesion Day 2. There was no significant correlation between tissue loss and time to eat ($r = 0.31$).

6-OHDA Lesions—In analyses including all rats, the magnitude of the dopamine depletion was found to be significantly correlated with each major pasta handling test variable (with asymmetry in adjustments: $r = 0.57$, time: $r = 0.61$, % contra as grasp paw/trial: $r = 0.53$, total atypical behaviors: $r = 0.75$, P 's < 0.02).

There was no significant effect of lesion side on the post-lesion reduction in contralateral adjustments in any study.

3.6. Video versus Real Time Results

Most data reported were collected in real time by the experimenter(s) counting left and right adjustments using hand tally counters. An advantage of the real time observations is that it is easier to rapidly change viewing position to optimize the view of the paws. An advantage of filming is that the tapes can be reviewed in slower speed. In the MCAO experiment, on days 0, 5 and 28, data were collected both in real time and, on the day before or after, separate trials were filmed and data collected in video playback by the same experimenter (author T.A.J.), who was experienced in the data collection. Video playback analysis included normal speed, slow motion and frame-by-frame analysis as needed to judge the occurrences of paw adjustments. Evaluation of one rat (4 trials) could typically be completed in 5 min or less. Averaged across all testing sessions, 9.39 ± 0.64 adjustments/paw/trial were recorded in real time in this group of animals and 10.15 ± 0.71 were recorded from video playback. In ANOVA for adjustments, there was no main effect of testing condition (video versus real time; $F(1,11) = 1.52, P = .24$). Compared to the pre-operative time point, animals with unilateral MCAO reduced the number of contralateral adjustments by 7.09 ± 2.72 per piece in real time and 9.38 ± 2.28 in video playback trials, as assessed on Day 5-6. In both real time and video, there were significant post-lesion reductions in adjustments by MCAO animals on Day 5-6 ($F's(1,11) = 17.06$ and 16.91) and Day 27-28 ($F's = 12.16$ and 4.82 , realtime and video, respectively, $P's < 0.05$) compared with pre-lesion. Thus, although slightly more adjustments may be obtained using video playback, the ability to detect lesion-induced changes was similar.

3.7. Experimenter Variability

Two experimenters collected all of the data from the intact animals reported in Section 3.1. When these animals were grouped by the data collector, the mean number of adjustments per piece recorded (left + right) was similar (16.16 ± 0.91 and 15.23 ± 0.77 , authors R.P.A. and T.A.J., respectively). Furthermore, when these experimenters rated the same videorecorded rats ($n=4$ intact and $n=5$ with MCAO), there was high correlation in the results. In these 9 rats, the total adjustments per trial recorded was 15.62 ± 2.87 (R.P.A.) and 16.12 ± 3.85 (TAJ), which was significantly correlated ($r = 0.92, F(1,7) = 37.7, P < 0.0005$). For time to eat per piece, R.P.A. recorded 17.65 ± 2.42 sec and T.A.J. recorded 16.69 ± 2.44 sec, which was also highly correlated ($r = 0.98, F(1,7) = 224.6, P < 0.0001$).

4. Discussion

Our purpose was to develop a quantitative measure of dexterous forepaw function in rats that would be both sensitive and easy to administer. Whishaw and Cole's (1996) detailed characterization of dexterous pasta handling in rats provided a strong foundation for this measure. We were interested in measures in which impairments in a single paw could be identified because upper extremity impairments are often lateralized. For these purposes, the measure of forepaw adjustments during vermicelli handling is suitable because it is quantitative, it revealed lateralized deficits, was sensitive to all lesion types examined here and because the data could be obtained with minimal effort and experience on the part of the experimenter. After SMC lesions, MCAO, and 6-OHDA lesions, there were enduring reductions in the adjustments made with the contra-to-the lesion forepaw.

Other vermicelli handling measures were useful for further characterizing the nature of the impairments and distinguishing between lesion types, as summarized in Table 3. Time to eat, adjustments with the ipsilateral forepaw and atypical behaviors were increased by 6-OHDA lesions. Atypical behaviors, but not eating time, were significantly increased in frequency at

one time point after MCAO (post-occlusion day 2). Neither time to eat nor the measured atypical behaviors were very sensitive to the effects of SMC lesions. Though significantly increased after MCAO, the expression of atypical behaviors was variable between rats and there was no single category of atypical behavior that was increased in most rats (in contrast to 6-OHDA effects). For all lesion types, the measure of adjustment number revealed consistent and enduring impairments. Thus, for documenting impairments and recovery, it may be that the measure of forepaw adjustments alone would be sufficient for many applications.

Many previous studies have found that CNS lesions can disrupt the movements used in food handling behavior in rodents (e.g., Castro, 1972; Kalil and Schneider, 1975; Kolb et al., 1977; Kolb and Holmes, 1983; Whishaw et al., 1992; Salamone et al., 1993; Whishaw and Coles, 1996; Whishaw et al., 1997; Gomez et al., 2006). The present results are largely consistent with these previous findings. After unilateral 6-OHDA lesions, Whishaw et al. (1997) counted forepaw movements during pasta eating, inclusive of movements on or near the pasta piece and postural adjustments. They found increased movements made by the ipsilateral paw in quantitative analysis and also observed that the contralateral paw made fewer adjustments, which seems similar to the effects of severe depletion in the present study. We further found that in rats with partial DA depletion the number of adjustments made by the contralateral paw was greatly reduced, whereas the number of compensatory ipsilateral paw adjustments was not increased. Salamone et al. (1993) also found that bilateral ventral striatal dopamine depletion greatly reduced the use of the forepaws for handling pellets during eating.

After aspiration of the sensorimotor cortex in rats, Whishaw and colleagues observed reduced digit movements in the contralateral paw during food handling (Whishaw et al., 1992) and increased abnormalities in grasping, as rated on a 1-4 scale (Whishaw and Coles, 1996). Kolb and Holmes (1983) have also found reduced digit use after motor cortical lesions.

The failure to find clear evidence for population bias (left versus right) in forepaw preferences for handling pasta in intact rats is consistent with studies of rat forelimb reaching behavior (Peterson, 1934; Whishaw, 1992). However, individual rats often had limb preferences for vermicelli handling behaviors. The majority (~77%) of rats displayed limb preferences for the grasp and guide roles. Additionally, slightly over half of the intact rats had a limb preference for forepaw adjustments, making 70% or more of all adjustments with one paw. After the SMC lesions, impairments in forepaw adjustments were more sensitively detected in rats with lesions contralateral to the preferred-for-adjustment limb.

There were significant positive correlations between asymmetries in forepaw adjustments and the severities of the MCAO and 6-OHDA lesions. However, this correlation did not reach significance after SMC lesions ($P = 0.054$). This is reminiscent of the lack of robust relationships between SMC lesion volumes and performance on skilled reaching tasks (Adkins et al., 2006; Metz et al., 2005). The SMC region includes several discrete subdivisions of the somatosensory and motor cortex (Donoghue and Wise, 1982) and it may be that the precise placement relative to these subdivisions is an important covariate, and this was not determined in the present study. This might be investigated in future studies, e.g., relating pasta handling behavior to the loss of movement representations that can be detected using intracortical microstimulation mapping. Despite this lack of significant correlation with lesion size, this measure of forepaw adjustments is clearly sensitive to the effects of SMC lesions given the failure of rats to completely recover normal contralateral adjustments within 45 days after the lesion.

Rats were not food deprived in the present analyses and this likely contributed to the failure to find slower eating after SMC lesions and MCAO, which is in contrast with several previous findings. For example, Whishaw and Coles (1996) found that SMC aspiration lesions increased

the time to eat angel hair pasta. In addition to the use of food deprivation, their analysis included stages preparatory to eating (e.g., picking up and flipping the pasta). The time to eat sunflower seeds was increased after cerebral ischemia in mice (Gomez et al., 2006) and after MCAO in rats (Gonzalez and Kolb, 2003), and these studies also used food restriction and included pre-consummatory behaviors in the time measurement.

It is important to note that the test described here is not intended to supplant careful descriptive analysis of food handling behavior. It could also bear further refinement for quantification of forepaw dexterity. The grasp and guide roles described in the present analyses are similar to the asymmetrical handling patterns described by Whishaw and Coles (1996), which they argued are variations of a power grip versus a precision grip. In the present study, both paws were found to make adjustments, but the specific movements used by the grasp versus guide paw have unique properties (Whishaw and Coles, 1996) and change during the course of eating, and these were not distinguished in the analysis. As mentioned, we did not quantify behaviors prior to placement of the pasta into the mouth, but abnormal behaviors preparatory to eating have been reported by Whishaw and others (1997) and were anecdotally noted in some rats in each lesion model in the present study. The test does not distinguish between haptic/somatosensory versus motor deficits. It is also likely to be influenced by oral motor impairments. In eating, movements of the paw are coordinated with movements of the mouth and at least some of the abnormalities found in the present study may be secondary to, or at least influenced by, sensorimotor abnormalities in the mouth, including the tongue (Whishaw et al., 1981) and this needs to be a consideration in the interpretation of the vermicelli handling results. Such impairments may have contributed to the abnormal angling of the pasta piece in rats with 6-OHDA lesions, a behavior previously described by Whishaw et al. (1997). However, oral-motor impairments seem unlikely to account for reductions in adjustments that are found in the contralateral but not ipsilateral forelimb.

In this study, MCAO was produced using intracerebral infusion of the vasoconstrictor, endothelin-1 (Sharkey & Butcher, 1995; Windle et al., 2006). This is not expected to be complicated by direct damage to muscles of mastication and swallowing, which can occur in the intraluminal filament model of MCAO (Dittmar et al., 2003). Given the possible contribution of oral motor impairments to the results, it would be important to control for any direct damage to muscles involved in eating when using this test to assay MCAO effects.

The test might also be extended to investigate the development and learning of skillful object manipulation. All rats used in the present study were made accustomed to vermicelli handling prior to the first tests. This was not merely to avoid neophobic responses. When rats are given a vermicelli piece for the first time, their handling of it is uncoordinated compared to a rat practiced in eating. Documenting how skill in pasta handling is developed with practice might be useful for investigations of bilaterally coordinated motor skill learning. Finally, the handling of a single type of food was investigated to narrow the problem in the present study, but it may be useful to now extend the measure of forepaw adjustment to pasta pieces of different lengths, diameters and shapes.

The primary paw-adjustment behavioral assessment is easy to carry out and, unlike skilled reaching tests, requires little specialized training of experimenters. Detecting the more subtle forepaw adjustments in real-time observations, however, does require some experience. In the present study, this was dealt with by having experimenters practice prior to data collection. Alternatively, the pasta handling sessions can be videotaped to be replayed in slower motion. Although counting adjustments in real time is a less meticulous approach, the results were reasonably similar to data collected from video playback for an experimenter practiced in the test.

Another methodological consideration is the angle of the experimenter's view of the paws. The dorsum of the paw, digits and knuckles were viewed in all trials because preliminary observations indicated that this enabled reliable detection of even subtle forepaw adjustments. This angle, however, was difficult to achieve with some of the 6-OHDA treated rats as a result of postural abnormalities. In fact, data from one rat had to be omitted from the analysis because the required view of the paws could not be achieved in the home cage or similar testing environment. This might be remedied by filming the rats from below; however, this requires further study to assess whether adjustment number varies with this shift in the angle of observation.

In our experience administering several different types of reaching tests (e.g., Adkins et al., 2006; Allred and Jones, in press; Maldonado et al., in press), the vermicelli handling test takes far less time and is more convenient. However, the tests are different in the quality and there remain significant advantages to the reaching tests as measures of skilled forepaw function. In reaching tests, each forepaw can be tested independently and measures that are largely insensitive to deficits in head and mouth movements are possible. The vermicelli handling test is a test of bilateral coordinated forepaw use that is sensitive to lateralized impairments but tests the two paws together. As noted above, the test is also likely to be influenced by deficits in head and mouth movements, such as may accompany MCAO and 6OHDA lesions. Reaching tests involve both proximal and distal forelimb movements and require learning to aim or learning reach trajectories, whereas, the measure of a vermicelli adjustments is primarily sensitive to distal forepaw function. Reaching tests are useful in models of motor skill learning and, as yet, the suitability of the vermicelli handling task for this purpose has not been tested.

In conclusion, the present manner of quantifying vermicelli handling behavior may be useful given the importance of modeling impairments in the use of the hands and the paucity of convenient tests of these impairments. Because it is simple to administer, it is reasonable to use this as one measure in a larger test battery. The measure of forepaw adjustments appears to be particularly sensitive to the effects of a wide range of injuries. This measure could undoubtedly be further refined, for example, to distinguish between categories of forepaw adjustments and to extend it to studies of skill development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A trial in which the rat displays a typical handling pattern. *A*, The vermicelli piece is dropped into a conveniently viewed part of the cage. *B*, The rat begins eating using an asymmetrical holding pattern. The left and right paws in this example are designated as “grasp” and “guide” paws, respectively. *C-D*, The paws move together as the piece becomes shorter and digits become interposed.

Figure 2.

Examples of adjustments. Each row is an example of the paw position before, during and after an adjustment. The adjusting paws are indicated by asterisks. *A-C*, Grasp-release-regrasp movements that reposition the paw on the pasta piece. *D-E*, Adjustments in which digits are partially extended and abducted followed by their flexion/adduction. In *E*, simultaneous adjustments in both paws are seen. Additional examples of adjustments can be found in supplementary material (VermiTutorial.ppt and VermiTrial.mov).

Figure 3.

Atypical and sporadic behaviors. *A, Paws together when long.* This symmetrical paw position is more typical of shorter pasta pieces. *B, Guide and grasp switch.* The right paw (arrow) initiates eating in the grasp position and switches to the guide position. *C, Failure to contact.* The right paw (arrow) fails to contact the piece. *D, Drop.* The arrow indicates the dropped piece. *E, Paws apart when short.* The left paw would typically move next to the right when the piece is this short. The arrow indicates the end of the pasta piece. *F, Mouth pulling.* The piece is held in the mouth and pulled through the paws as they slide down. *G, Abnormal posture.* The pasta piece rests on the floor and the head is brought down to it as it is eaten. *H, Iron grip* and *I, Guide around grasp.* The grasp paw (arrow) is held in an iron grip, while the guide paw (as defined at the start of eating) moves around it. *J, Angling with head tilt.* The pasta piece is angled towards the intact body side as the head is tilted. The right paw is being held in an iron grip. The last three categories are characteristic of unilateral 6-OHDA lesions.

Figure 4. Representative coronal sections of *A*, an ischemic SMC lesion, *B*, an electrolytic SMC lesion and, *C*, a lesion resulting from MCAO. Numbers to the right are approximate coronal plane distances relative to bregma in mm. Scale bar = 1 mm

Figure 5.

Ischemic SMC lesion effects on vermicelli handling. *A*, Adjustments made with the contralateral forepaw decreased compared to sham-operates and this endured throughout the 45 day post-lesion period of testing. Day 0 is the pre-operative time point. Electrolytic SMC lesions resulted in similar effects (see text for details). * $P < 0.05$ significantly different from sham. *B*, The magnitude of the post-lesion change in adjustments varied with endogenous asymmetries. Lesion effects in contralateral adjustments were less sensitively detected in rats that preferred to use the unimpaired forelimb for adjustments prior to the lesion (Ipsi Bias subgroup). † $P < 0.05$ significantly different from Contra/No Bias subgroup. *C*, Adjustments

with the ipsilateral forelimb, time to eat and atypical behaviors were not significantly changed after the lesion.

Figure 6.

MCAO effects on vermicelli handling. Lesions were made contralateral to the paw that was used most for adjustments in preoperative tests. **A**, Unilateral MCAO resulted in an enduring decrease in adjustments made with the contralateral forepaw. $*P < 0.05$, significantly different from Day 0. (Note the difference in scale compared with Fig. 5.) **B**, There were no significant changes in ipsilateral adjustment number or time to eat. **C**, Atypical behaviors were increased. This was significant relative to the preoperative time point at Day 2 ($*P < 0.05$). Data shown for Days 0, 5 and 28 are the average of 2 days, one collected in real time and the other from video playback (see text for details).

Figure 7.

6-OHDA lesion effects on vermicelli handling. *A*, Both partial and severe dopamine depletions reduced contralateral adjustments compared with normals. After severe depletions, there was also a major *increase* in adjustments made with the ipsilateral forepaw. *B*, The time to eat was increased after severe depletions. *C*, Atypical behaviors were increased in both depletion groups. After severe depletion most increased were behavioral categories 8-10, iron grip, angling with head tilt and guide around grasp (see Fig. 3H-J). Error bars are for total atypical observation number (summed over categories 1-10). * $P < 0.01$ compared with normal.

Table 1
Striatal Catecholamine Content of Rats used in the unilateral 6-OHDA study

Depletion Group	DA $\mu\text{g}/\text{mg}$	% DA remaining	DOPAC $\mu\text{g}/\text{mg}$	% DOPAC remaining	HVA $\mu\text{g}/\text{mg}$	% HVA remaining
Normal (n=12)	11 $\pm$ 1.1	80 $\pm$ 5	1.1 $\pm$ 0.12	87 $\pm$ 9	0.59 $\pm$ 0.05	84 $\pm$ 4
Partial (n=5)	5.8 $\pm$ 3.0 *	30 $\pm$ 15 **	0.57 $\pm$ 0.29 *	32 $\pm$ 2 *	0.35 $\pm$ 0.14 **	37 $\pm$ 14 *
Severe (n=8)	0.23 $\pm$ 0.04 **	1.5 $\pm$ 0.3 **	0.09 $\pm$ 0.00 **	6.7 $\pm$ 0.8 **	0.036 $\pm$ 0.009 **	4.1 $\pm$ 0.7 **

Data are means $\pm$ SEM content per wet tissue weight in the depleted hemisphere and the % depleted/contralateral striatal content.

* $p < 0.02$;

** $p < 0.0002$ significantly different from normals (failed and sham groups). There were no group differences in DA, DOPAC and HVA contents in the striatum contralateral to the depletion.

Table 2
Atypical Behaviors in Rats with 6OHDA Lesions

Atypical/Sporadic Behavior	Normal	Percent of Rats	
		Partial Depletion	Severe Depletion
1. Paws together when long	50	100 [*]	12.5
2. Guide and grasp switch	33	80	50
3. Failure to contact	8.3	0	12.5
4. Drop	0	20	37.5 [*]
5. Paws apart when short	0	20	12.5
6. Mouth pulling	0	0	0
8. Iron grip	0	20	87.5 ^{**}
9. Guide around grasp	0	20	87.5 ^{**}
10. Angling with head tilt	0	20	87.5 ^{**}

Data are the % of rats showing the behavior in at least one trial.

*
 $p < 0.05$,

**
 $p < 0.001$ significantly different from Normal in chi-square analysis.

Table 3

Summary of Lesion-Induced Changes in Vermicelli Handling Behaviors

Variable	SMC Ischemic	SMC Electrolytic	MCAO	6OHDA Partial	6OHDA Severe
Contra Adjustments	↑↑	↑↑	↑↑	↑↑	↑↑
Insi Adjustments	=	=	=	=	↑↑
Time to Eat	=	=	=	=	↑↑
Atypical Behaviors	=	↑	↑↑	↑↑	↑↑
Contra as Grasp Limb	=	=	=	↑	↑
Insi as Grasp Limb	=	=	=	↑↑	↑↑

Symbols: Mean difference relative to controls or pre-lesion values: up arrows, increased; down arrows, decreased; equal sign, no significant difference; double up arrows, two fold or greater increase; double down arrows, half or less.