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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 previously described the dexterous manner in which rats manipulate food items with their paws, including thin pieces of pasta [Whishaw IQ, Coles BL. Varieties of paw and digit movement during spontaneous food handling in rats: postures, bimanual coordination, preferences, and the effect of forelimb cortex lesions. Behav Brain Res 1996;77:135–48]. 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

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 and Jones, 2008; Maldonado et al., in press). However, they tend to be labor intensive to administer, which may sometimes preclude

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their use as one of a large battery of tests. They require specialized apparati, 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., 1998, 1992; Ivanco et al., 1996; Whishaw and Coles, 1996). 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-h light:12-h 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