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Research report

Cineradiographic (video X-ray) analysis of skilled reaching in a single pellet reaching task provides insight into relative contribution of body, head, oral, and forelimb movement in rats

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ABSTRACT

The forelimb movements (skilled reaching) used by rats to reach for a single food pellet to place into the mouth have been used to model many neurological conditions. They have been described as a sequence of oppositions of head–pellet, paw–pellet and pellet–mouth that can be described as movements of the distal portion of body segments in relation to their fixed proximal joints. Movement scoring is difficult, however, because the location and movement of body segments is estimated through the overlying fur and skin, which is pliable and partially obscures movement. Using moderately high-speed cineradiographic filming from lateral, dorsal, and frontal perspectives, the present study describes how forelimb and skeletal bones move during the skilled reaching act. The analysis indicates that: (i) head movements for orienting to food, enabled by the vertical orientation of the rostral spinal cord, are mainly independent of trunk movement, (ii) skilled reaching consists of a sequence of upper arm and extremity movements each involving a number of concurrent limb segment and joint movements and (iii) food pellets are retrieved from the paw using either the incisors and/or tongue. The findings are discussed in relation to the idea that X-ray cinematography is valuable tool for assisting descriptive analysis and can contribute to understanding general principles of the relations between whole body, head, oral, and upper extremity movement.

1. Introduction

Use of a forelimb to reach for food, conventionally called skilled reaching, is an evolutionary old movement, displayed widely among terrestrial vertebrates [17]. Rodents and primates belong to sister clades and share many similarities in their skilled movements. Therefore, the laboratory rat and mouse are useful for investigating basic neural questions related to skilled movements [5,7,18,19,21,27,30] and serve as animal models to study motor system injury due to stroke [3,7,34], trauma [36], spinal cord injury [12,19], and a number of neurological disorders including Huntington's chorea [9] and Parkinson's disease [22]. Skilled reaching in normal and experimental model rats has been contrasted using at least three classes of measurement: end point measures, including measures of reach successes and reach attempts [29], Cartesian representations of the movement of the distal ends of various body segments [32], and taxonomic measures derived from movement notation techniques [1,28,35]. The notation techniques describe the movement of the distal portions of limb segments in relation to their fixed proximal portion and so capture the individual contributions of many simultaneously moving body segments. The similarities in movement changes in animal models of human disease attest to the utility of these analytical measures.

In most studies using the rat, the movements of reaching have been described from video recordings. A drawback of the method is that whereas the distal end of the paw can be clearly tracked, representations of the proximal and distal ends of other body segments are more difficult to make. Markers are not easily attached to the overlying skin and the movement of the skin as a consequence of limb or trunk movements makes the location of joints difficult. Cineradiographic/video X-ray analysis provides a solution to this problem. Cineradiography gives an X-ray image of the skeleton as an animal performs the reaching task and thus provides a way of documenting skeletal movements. A number of studies have used cineradiography to describe locomotion [8,15,23,26] and one study has examined reaching in the cat [4]. No previous studies have used cineradiography as a method to measure movements of

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the rat or to confirm notational scoring systems used for the rat. The present study uses cineradiography to address a number of questions, including whether orienting of the head to locate food is independent from trunk movement, the extent to which movements of different limb segments are sequential and concurrent, and how the rat uses its mouth to remove food from its paw.

The rats were viewed using cineradiography or were filmed with video as they performed a single pellet reaching task. Reaching movements from both methods were compared frame-by-frame. The findings are discussed in relation to: topography of skeleton during skilled reaching and the utility of cineradiographic methods for understanding general principle of skeleton organization and movement.

2. Materials and methods

2.1. Subjects

Four female (≈100 days old, with body weight 270–310 g) Long-Evans hooded rats from the University of Lethbridge and four female rats of the same strain (≈100 days old, with body weight 296–317 g) from the University de Montreal animal colony were used. The experiments were conducted in compliance with the guidelines of the animal care committees of the University of Lethbridge and the University of Montreal as outlined by the Canadian Council for Animal Care, which complies with international standards for animal care. Rats were housed in Plexiglas cages (36 cm long, 20 cm wide, and 21 cm deep) with sawdust bedding, in groups of two or three in a colony room maintained on a 12-h light:12-h dark cycle (08:00–20:00 h) with controlled temperature and humidity.

2.2. Feeding and food restriction

Prior to and during training, the rats were gradually food deprived to 90–95% of their body weight by once a day feedings of 18–20 g of Purina rat chow. The week prior to training, each rat received twenty 45 mg dustless precision banana-flavored pellets (product #F0021, Bioserve Inc., Frenchtown, NJ, USA) 1 h prior to the daily chow ration. The pellets would later serve as reaching targets. Once reach training began and until the end of the study only rat chow was served in the home cage.

2.3. Single pellet reaching box

The single pellet reaching box [28] is made of clear Plexiglas with the dimensions 35 cm long × 14 cm wide × 45 cm high. For X-ray imaging the reaching box has the same dimensions except the height (30 cm) to position the amplifier close to the rat to improve X-ray images. At the center of each front wall of the box there is a vertical slot, 1 cm wide extending from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slot, a 2 cm deep shelf is mounted 3 cm above the floor. Two indentations on the surface of the shelf are located 2 cm from the inside of the wall in order to hold the food targets. The indentations were aligned with the edges of the slit. This location prevents a rat from lapping the food with its tongue, and because the paw pronates medially to grasp, requires the use of the contralateral paw for retrieval.

2.4. Recording apparatus

2.4.1. Video recording

Reaching performance was recorded using a Sony 3CCD camcorder (shutter, speed 1/1000 s) and a cold light source. A Sony videocassette recorder DSR-11 was used for subsequent frame-by-frame analysis (30 frames/s).

2.4.2. X-ray recording

Cineradiographic recording used a high-speed motion analysis camera hooked to an X-ray machine (Siemens Coroskop Bicor) as illustrated in Fig. 1. Continuous X-rays (100 kV, 8–9 mA) were applied during the reaching movement of the rats while digital images were acquired at 120 frames/s either in the lateral, frontal or dorsal perspective with a camera (DALSTAR CA-D6, Dalsa Corp., Ontario, Canada) directly mounted on the 27 cm diameter image intensifier. In order to freeze the movements without compromising the quality of the image, the shutter speed was set at 1/500 s. These digital images were directly recorded on a PC using VisionNow Imaging System [Boulder Imaging, Louisville, CO, USA; http://www.boulderimaging.com].

2.5. Training

The training consisted of daily 15–20 min sessions for each rat. The first objective was to introduce the rat to the reaching cage with pellets on the shelf and to have the rat retrieve the pellet by paw or tongue. Once a rat was successfully retrieving the pellet, pellets on the shelf were moved further away in order to encourage the use of a paw. After a rat demonstrated a preference for one paw, by making more reaching attempts with it, individual pellets were placed into the indentation contralateral to that paw. During the second week, rats continued to receive daily 20 min training sessions, consisting of discrete trials with inter-trial intervals during which the rats were shaped to leave the slot, walk to the rear wall of the cage, turn and approach the slot again for the next pellet. In addition, by withholding food on semi-randomly selected trials, rats were taught to sniff the shelf for a pellet and to reach only if a pellet was present. Thus, each rat eventually learned to orient to the food pellet, transport its limb through the slot, grasp the food pellet, and retract its paw through the slot to release the food into its mouth [11]. Over days, the rats were trained with different orientation of the cage relative to experimental room. In addition, the rats to be used for cineradiography were acquainted to the room and the sounds related to X-ray recording and equipment prior to the formal tests.

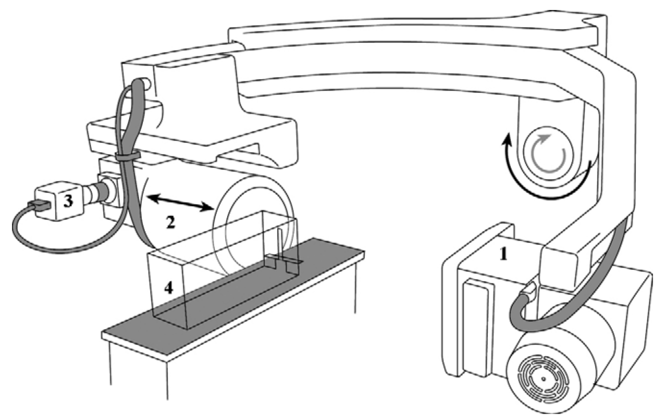

Fig. 1. Experimental setup. The number corresponds to: 1, X-ray source; 2, image amplifier of X-ray system; 3, camera recording the X-ray sequence from the image amplifier; 4, reaching cage. Bidirectional arrow indicates to movement range of the image amplifier for appropriate zooming of the rat or forelimb segments; curved arrows – range of motion of X-ray system to mount the X-ray source and image amplifier in desired position for filming the rats either from lateral, dorsal or frontal perspective.

2.6. Timeline

After achieving 50% in total success in the reaching task the rats were recorded by conventional video and X-ray cinematography techniques. Reaching performance (success level) was scored only during training period but not during recording of the reaching movements. In separate blocks of trials video camera or X-ray source and amplifier was mounted either laterally, dorsoventrally or frontally to obtain images of the rats' forelimb and head in the vertical and anteroposterior directions, anteroposterior and mediolateral directions or vertical and mediolateral directions, respectively.

2.7. Behavioral analysis

2.7.1. Quantitative analysis

Reaching behavior was assessed by the following measures:

(1) *Total success*. A successful reach was defined as one in which an animal grasped a food pellet, transported it with the paw into the cage, and placed it into its mouth. To achieve a successful reach, the rat was free to advance the paw toward the food in as many "tries" as required. Total success was calculated as: success% = number of pellets obtained/20 × 100;
(2) *First trial success*. First trial successes were those in which a rat obtained a food pellet on the first advance of the limb toward the food as calculated as: success% = number of pellets obtained on first advance/20 × 100.

2.7.2. Movement pattern analysis

The various movements comprising a reach have been identified using a conceptual framework derived from Eshkol–Wachmann Movement Notation (EWMN) [6,28]. In brief, EWMN is designed to express relations and changes of relation between the parts of the body. The body is treated as a system of articulated axes, i.e. body and limb segments. A limb is any part of the body that either lies between two joints or has a joint and a free extremity. These are imagined as straight lines (axes), of constant length, which move with one end fixed to the center of a sphere. An important feature of EWMN is that the same movements can be notated in several polar coordinate systems. The coordinates of each system are determined with reference to the environment, to the animal's body midline axis, and to the next proximal or distal limb or body segment. By transforming the description of the