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# A system for automated tracking of motor components in neurophysiological research

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ABSTRACT

In the study of motor systems it is often necessary to track the movements of an experimental animal in great detail to allow for interpretation of recorded brain signals corresponding to different control signals. This task becomes increasingly difficult when analyzing complex compound movements in freely moving animals. One example of a complex motor behavior that can be studied in rodents is the skilled reaching test where animals are trained to use their forepaws to grasp small food objects, in many ways similar to human hand use. To fully exploit this model in neurophysiological research it is desirable to describe the kinematics at the level of movements around individual joints in 3D space since this permits analyses of how neuronal control signals relate to complex movement patterns. To this end, we have developed an automated system that estimates the paw pose using an anatomical paw model and recorded video images from six different image planes in rats chronically implanted with recording electrodes in neuronal circuits involved in selection and execution of forelimb movements. The kinematic description provided by the system allowed for a decomposition of reaching movements into a subset of motor components. Interestingly, firing rates of individual neurons were found to be modulated in relation to the actuation of these motor components suggesting that sets of motor primitives may constitute building blocks for the encoding of movement commands in motor circuits. The designed system will, thus, enable a more detailed analytical approach in neurophysiological studies of motor systems.



## 1. Introduction

The central nervous system fundamentally deals with the control of actions. Consequently behavioral studies have often been a natural starting point for investigations aimed at understanding its functions. For the same reason, the search for new therapies for neurological and psychiatric diseases largely depend on animal models designed to mimic certain aspects of the disease that cause observable changes in the behavior of the subject. With the more recent development of techniques allowing for simultaneous recording of neuronal activity in many parts of the central nervous system in freely behaving animals, the electrophysiological processes underlying such changes in behavior – or even the generation of specific components of observed actions – have the potential to be investigated in much greater detail (Nicolelis, 2008). The access to neuronal data with sub-millisecond temporal precision in turn further increases the need for more detailed documentation of movement patterns displayed by freely behaving animals. However, because natural behavior typically involves chains of movement sequences incorporating many partially overlapping motor components, an extra challenge in this respect is to reliably identify and isolate the execution of these individual motor elements. The most common approach for behavioral recording in neurophysiological research is probably the use of digital video techniques, where image sequences are obtained from different camera angles and specific behaviors are either manually identified off-line (Cenci and Lundblad, 2007; Whishaw et al., 1999) or, when clearly visible in any of the cameras, automatically identified and quantified from this viewing angle (Peikon et al., 2009; Vorhees et al., 1992). In situations where movements involve several joints the problem of tracking motor components involving for example small angle changes in distally located joints becomes increasingly complex. A well-studied and functionally very important example of movement sequences involving parallel movements in multiple joints in humans is the skilled arm and hand movements involved in reaching for and grasping of different objects (de Bruin et al., 2008; Gentilucci et al., 1997; Jeannerod, 1984). Perhaps surprisingly, rodents can after extensive training also perform reaching and grasping movements using their forepaw in many ways similar

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to a human hand, hence making this behavior particularly well suited for studies on skilled motor control in translational research (Peterson, 1934; Sacrey et al., 2009).

To be able to track forelimb movements with high fidelity in the skilled reaching task we designed a system that uses a three-dimensional (3D) model of the paw for which movements are reconstructed based on image sequences recorded from multiple viewing angles. Each paw pose is estimated by an optimization procedure that maximizes a matching quality measure in order to retrieve the best approximation of that pose. The matching quality is measured as the discrepancy between projections of the 3D model onto the image planes and the actual images, using edges and silhouettes as cues. We here describe how this system allows us to correlate single unit activity of neurons in corticostriatal circuits in rats to different motor components in the reaching-grasping sequence, opening up for significantly more detailed analyses of skilled movement control.

## 2. Materials and methods

### 2.1. Animals

One adult female Sprague–Dawley rat (230 g; Taconic Inc.) was used in the study. The animal was kept on 12:12 h light cycle and received food and water ad libitum except for a 22 h-period prior to each testing session during which no food was provided. After each testing session the animal had free access to food for 1 h. All experiments were approved in advance by the Malmö/Lund ethical committee of animal experiments.Training protocol

The rat was trained during a three week period prior to the implantation of recording electrodes according to the protocol described by Whishaw et al. (2008). Training entailed habituation of the rat to the apparatus, habituation to the food reward (pellet) and establishment of paw dominance. The training continued until performance no longer improved between sessions, reflecting a fully learned behavior (Hermer-Vazquez and Moshtagh, 2009). See Appendix A for details.

### 2.3. Electrodes

Formvar-insulated 33 μm tungsten wires (CFW Inc.) were arranged into four separate $4 \times 5$ arrays with 250 μm spacing between adjacent wires. Each array consisted of 16 recording channels and two reference channels, as well as two blind channels. The wires of each array were cut to the appropriate length for the corresponding recording site (cortical or striatal). Reference wires were cut ∼1 mm shorter than the recording wires and deinsulated ∼300 μm at the tip, positioning them dorsally to the recording site (at the cortical surface and within the corpus callosum, for cortical and striatal arrays, respectively). The wires were attached to board-to-board-connectors (Kyocera 5602) with conducting epoxy (Epotek EE 129-4), and linked to the acquisition device via a board-to-Omnetics connector adapter (Kyocera 5602; Omnetics). A 200 μm silver wire was used as animal ground via direct connection to four screws inserted into the cranium.

### 2.4. Surgery

Implantations were performed under Fentalyl/Medetomidine anesthesia (0.3/0.3 mg/kg, i.p.). Electrodes were implanted in the forelimb area of the primary motor cortex (center coordinates: AP: +1.5, ML: ±2.8, DV: −1.0 from cortical surface, Donoghue and Wise, 1982) and of the striatum, (center coordinates: AP: +0.2, ML: ±3.8, DV: −3.5 from cortical surface, West et al., 1990) in both hemispheres. The implant was fixated with dental acrylic attaching to screws in the skull. After surgery the anesthesia was reversed by Atipamezole hydrochloride (5 mg/kg, i.p.). Buprenorphine (0.5 mg/kg, s.c.) was administered as postoperative analgesic. The animal was allowed to recover for a week after implantation before testing commenced.

### 2.5. Experimental set-up

The testing apparatus for the reaching task consisted of a 450 mm × 140 mm × 350 mm (l/w/h) transparent Plexiglas cage with a 13 mm wide aperture at the middle of one of the short sides (vertical position of aperture: 40–150 mm above the ground). Outside the aperture a 30 mm deep shelf was positioned. To facilitate the placement of food pellets, three separate hemispherical indentations (5 mm in diameter) were made in the shelf 15 mm from the outer edge of the slit. The middle pocket was positioned right in front of the aperture with the other two pockets centered 6.5 mm more lateral on each side. This configuration prevented the rat from using its tongue to acquire the pellet. Furthermore, it permitted the experimenter to decide which paw the rat had to use, as this geometry allows only reaches with the paw contralateral to the side pockets (for further details, see Whishaw and Pellis, 1990). At the center of the cage was a 40 mm high solid obstacle that enforced a forelimb stepping movement similar to the actual reaching and grasping movement, for comparison of similar movements with different purposes.

### 2.6. Reaching task

In the behavioral task the rat was placed in the reaching apparatus from where it could obtain 45 mg food pellet rewards, positioned in the indentation on the reward shelf, via controlled reaches through the aperture of the wall. A trial ended either if the rat acquired the pellet after one or several reach attempts, or if the pellet at any time was moved from its original position, in which case the food pellet was manually removed by the experimenter. In order to produce discrete reaching trials, the rat was trained to return to the end of the cage opposite to the reaching slit before it was presented with another food pellet, requiring the animal to reposition before every trial. Moreover, by semi-randomly withholding food the rat was prompted to identify the presence of a pellet before each reach attempt, yielding maximal accuracy of each skilled reach (Appendix A).

### 2.7. Acquisition of neurophysiological signals

Extracellular neuronal recordings were acquired using a multichannel recording system with Cheetah software (Neuralynx Inc.) and digitized at 32 kHz per channel. Local field potentials were bandpass filtered between 0.1 and 300 Hz (not used in this study) and action potential waveforms between 600 and 9000 Hz.

### 2.8. Video acquisition systems

The details of the paw movement during the reach and grasp behavior were captured by two front-view cameras (CMOS, 640 × 480 pixels; Dalsa Inc.) positioned close to the aperture. Additionally, three mirrors positioned along the edges of the reward shelf gave two extra viewing planes for each front-view camera. Thus, the front-view cameras and mirrors were mounted such that six complementary viewing planes covered the region of interest where the rat forelimb was moving. Extra care was taken to avoid uneven light conditions or reflexes from surfaces. To ensure that inadvertent variations in the camera positions would not influence the 3D reconstruction a calibration procedure with an object of known measures was performed at the start of each recording.