Neuroscience 147 (2007) 573–582

REACHING AND GRASPING PHENOTYPES IN THE MOUSE (*MUS MUSCULUS*): A CHARACTERIZATION OF INBRED STRAINS AND MUTANT LINES

V. TUCCI,[a]* F. ACHILLI,[a] G. BLANCO,[a] H. V. LAD,[a] S. WELLS,[b] S. GODINHO[a] AND P. M. NOLAN[a]

[a]*MRC Mammalian Genetics Unit, Harwell, Didcot, Oxfordshire OX11 0RD, UK*

[b]*MRC Mary Lyon Centre, Harwell, Didcot, Oxfordshire OX11 0RD, UK*

Abstract—Skilled movements, such as reaching and grasping, have classically been considered as originating in the primate lineage. For this reason, the use of rodents to investigate the genetic and molecular machinery of reaching and grasping has been limited in research. A few studies in rodents have now shown that these movements are not exclusive to primates. Here we present a new test, the Mouse Reaching and Grasping (MoRaG) performance scale, intended to help researchers in the characterization of these motor behaviors in the mouse. Within the MoRaG test battery we identified early phenotypes for the characterization of motor neurone (Tg[SOD1-G93A]^{dl}1Gur mice) and neurodegenerative (TgN(HD82Gln)81Dbo transgenic mice) disease models in addition to specific motor deficits associated with aging (C3H/HeH inbred strain). We conclude that the MoRaG test can be used to further investigate complex neuromuscular, neurological, neurodegenerative and behavioral disorders. Moreover, our study supports the validity of the mouse as a model for reaching and grasping studies.

Key words: reaching, grasping, motor phenotypes, mouse, inbred strains, mouse mutants.

From a behavioral perspective, the investigation of movement is far more complex than studying the multi-joint architecture of the musculoskeletal system given that many different regions of the brain control the movement. Reaching and grasping are two fundamental goal-directed movements. By reaching and grasping within our space, we are able to interact with the environment, achieving goals and showing intentions. Motor impairments, including those inherited or acquired through life events and aging, can affect a considerable portion of the human population. A broad spectrum of genetic defects may account for physical impairment (including weakness and poor muscle control) in skilled movements. For example, Parkinson's disease (PD) patients often manifest difficulties in coordinating actions of the hand (e.g. handwriting (Van Gemmert et al., 2001)), show impairment in their ability to synchronize the arm, the hand and the torso (Bertram et al., 2005) as well as deficits in controlling hand opening and closing during reach-to-grasp movements (Rand et al., 2006).

Through the development of genetic tools, the mouse has become a crucial organism to model human disorders. Recombinant DNA technologies, which have enabled the generation of mice carrying human disease allele-mutations, when combined with the detailed analysis of phenotypes can aid in defining the onset and progression of disease symptoms. Parameters/tests such as grip strength, placing response, rope grip test, isometric resistance, electromyography, open field, swim speed, gait measurement and rotarod are all amenable motor function tests in the mouse (see (Tucci et al., 2006) for an overview) and are mainly used to assess disease progression in neurological and neurodegenerative mouse mutants (Carter et al., 1999; Fernagut et al., 2002; Wooley et al., 2005). Nevertheless, commonly-used mouse motor tests do not account for the more skilled aspects of motor function in rodents such as manipulative ability, climbing, reaching and grasping movements. Traditionally, most research related to these skilled behaviors has been conducted in primates since rodents have not been considered to be an applicable model (Napier, 1980; Passingham, 1982; McNeilage, 1990; Canedo, 1997). As a consequence rodents have been ignored in the investigation of motor skilled behaviors. Contrary to these classical beliefs, recent studies in rats have questioned this established viewpoint and have shown that reaching and grasping are structurally, anatomically and functionally similar in rodents and primates (see (Whishaw, 2003) for an overview). Generally, these analyses (Farr and Whishaw, 2002) rely on adaptations of human movement scoring systems and involve laborious scrutiny of video recordings at a high temporal resolution making them unamenable to the systematic analysis of mouse strains and mutants. In developing the staircase test, Montoya et al. (1991) provided a quantitative measure of reaching performance in rats, later repeated in mice (Baird et al., 2001). Although the test is reliable, it is limited in its ability to define multiple reaching and grasping parameters and can take up to several weeks to complete.

Here we present a novel behavioral test for the quantitative and qualitative assessment of reaching and grasping motor parameters in the mouse, the Mouse Reaching and Grasping (MoRaG) performance scale. As a complement to the studies above, the test was developed as a high-throughput screen for the analysis of skilled motor function and has proven to be successful in detecting skilled movement differences in mouse strains and mu-

*Corresponding author. Tel: +44-01235-841194; fax: +44-01235-841200.
E-mail address: v.tucci@har.mrc.ac.uk (V. Tucci).
Abbreviations: ALS, amyotropic lateral sclerosis; ky, kyphoscoliosis; LFR, latency to the first reach; *Loa*, legs at odd angles; MoRaG, Mouse Reaching and Grasping; RPE, right paw entry.

0306-4522/07$30.00+0.00
doi:10.1016/j.neuroscience.2007.04.034

tants. The test is also a suitable phenotyping procedure for large-scale mutagenesis studies (for example (Nolan et al., 2000)).

EXPERIMENTAL PROCEDURES

Animals and husbandry

The study was conducted at the MRC Mammalian Genetics Unit, Harwell (UK). All animal studies described in this paper were carried out under the guidance issued by the Medical Research Council in "Responsibility in the Use of Animals for Medical Research" (July 1993) and Home Office Project License No. 30/2198. All experiments conformed to international guidelines on the ethical use of animals. All efforts were made to minimize the number of animals used and their suffering. Mice were bred in-house and were weaned (five per cage) into IVC cages (Techniplast, London, UK) enriched with sawdust, shredded tissue (Datesand, Manchester, UK) and the addition of a cardboard fun tunnel (Datesand). Access to an expanded breeder diet of food nuts (Special Diet Services, Witham, Essex, UK) and water (chlorinated between 15 and 20 parts per million) was *ad libitum* prior to and following the test (see details of test below). Additional conditions that remained uniform were as follows: temperature (between 19 and 23 °C), humidity (45–65%), and 12-h light/dark cycle with lights on at 07:00 h. Unless otherwise indicated, all tests were carried out on male mice between 8 and 10 weeks of age and six mice per genotype were used.

Wild-type mice. We subjected C57BL/6J, BALB/cByJ, C3H/HeH, FVB/NCrlBr, 129/SvPas, 129/SvNiMR and 129/SvEv mice to one MoRaG test session. Furthermore we tested four aged C3H/HeH males (2 years old). In addition, we used 10 F1 (C57BL/6J×C3H/HeH) mice in an extended protocol to assess motor learning. Mutant lines were chosen as they exhibited deficits in a variety of features that could affect skilled motor function. *Neuromuscular mutant mice:* Heterozygous and homozygous kyphoscoliosis (*ky*) mice and BDLM controls have been subjected to the MoRaG test. The kyphoscoliotic-*ky* mouse is a classical spontaneous mutant that suffers from muscular dystrophy leading to secondary onset of spinal deformity (Bridges et al., 1992; Zheng et al., 1999). Homozygote animals develop symptoms from weaning, including postural changes and a defective placing response, whereas heterozygote animals appear to be unaffected. Two additional novel mouse mutants identified within the Harwell ENU mutagenesis program with deficits in neuromuscular function were tested (Nolan et al., 2000). The ostes line is a semidominant mutation that displays a tremor phenotype when suspended by the tail with muscle wasting and muscular dystrophy (http://andy.emma.cnr.it/jEmma/strains/strain_187.utf8.html). Heterozygote ostes mice do not develop a tremor phenotype and show only mild muscle pathology. Heterozygous and homozygous mice were tested using the MoRaG test. *Motor neuron mutant mice*: The "legs at odd angles," *Loa*, mutant line arose in an ENU mutagenesis screen and exhibits a dominant cramping phenotype when raised by the tail with a progressive deterioration of neurological parameters detectable from 1 month of age (Rogers et al., 2001). *Loa* mice carry a missense mutation in the cytoplasmic dynein heavy chain and display a progressive motor neuron degeneration (Hafezparast et al., 2003). Heterozygous mutant and wild-type littermate controls were tested. In addition, we tested mice transgenic for the human SOD1 G93A mutation, Tg[SOD1-G93A]^{dl}1Gur, along with littermate controls. The Tg[SOD1-G93A]^{dl}1Gur subline, which has approximately 30% fewer copies of the transgene construct than the high copy number line (Gurney, 1994), represents a valuable mouse model of familial amyotropic lateral sclerosis (ALS). Mutants become paralyzed in one or more limbs from about 6–7 months of age. *CNS neurodegenerative mutant mice*: We tested four transgenic mice that express an N-terminally truncated huntingtin cDNA containing 82 glutamines and encompassing the first 171 amino acids of huntingtin (TgN(HD82Gln)81Dbo) and five wild-type littermate controls. TgN(HD82Gln)81Dbo mice have previously shown behavioral and pathological abnormalities resembling those of Huntington's disease, HD (Schilling et al., 1999). Finally, a dominant ENU-induced mutant line, moonwalker (http://andy.emma.cnr.it/jEmma/strains/strain_431.utf8.html), was tested with littermate controls. Moonwalker mice develop an ataxic gait from weaning and show an associated loss of cerebellar Purkinje cells.

MoRaG test

The MoRaG apparatus consists of a custom-made Plexiglas chamber 10.5 cm high by 7.5 cm deep by 6 cm wide (Fig. 1). On the outside front wall of the chamber, a feeding platform, accessible through a 6 cm wide opening, is located 5.5 cm from the floor. Mice are primed to perform in the task by restricting access to food for up to16 h before the task. Prior to commencing the task, mice were placed in the apparatus for a 5-min acclimatization period. To commence the task, small food pellets were placed at approximately 1.5 cm distance on the feeding platform. As the opening is not large enough for the mouse to collect the food pellet using its mouth (nose poking behavior), the mouse eventually used one or both forelegs. Each session consisted of 30 consecutive trials lasting approximately 15–20 min in total. The motivation of the mouse to perform a foreleg movement for several trials is something we considered while designing the MoRaG protocol since it may affect the motor performance. Within a set of preliminary experiments (not reported here) we observed that all the wild type mice were able to perform at least 50–60 consecutive trials using small pellets. However, this can vary in particular strains and mutants and therefore we reduced the number of trials. We are also aware that the 16-h food restriction protocol employed in our study is mild (see (Tucci et al., 2006)). In countries other than UK the restriction protocols may last longer and it is likely that this would increase the motivation of animals. Each trial began when the pellet was delivered to the feeding platform and ended when the mouse collected it. The inter-trial interval varied between 10 and 20 s. After the test all the mice were returned to the home cage and provided with food *ad libitum*.

Performance in the task was assessed using 19 semiquantitative and five quantitative parameters (see Table 1 for a complete list of the parameters). Semiquantitative parameters were selected from a wide range of possible abnormalities that may affect neurological, neuromuscular and behavioral function (e.g. tremor, speed of movement, body and shoulder position, posture etc.) and were measured in four domains. 1) Spontaneous behaviors such as general activity, defecation and grooming were recorded during acclimatizing. During this phase, the general adaptation of the animal to the novel condition was assessed. 2) Outward phase behaviors were recorded during the phase of target-directed movement (reaching). 3) Reversal phase behaviors were recorded during the preparation for and execution of grasping. 4) Inward phase behaviors were recorded during retrieval and consumption of the food pellet.

A series of quantitative behaviors was also recorded. 1) Reaction times to reach the food pellets, 2) reaching accuracy, 3) grasping accuracy and 4) right paw entries, RPE (Collins, 1968, 1969). The reaction time between when the mouse sees and reaches the food pellet (reaching time) is considered for all the trials. The latency to the first reach (LFR) is an indication of motor flexibility. The qualitative parameters have been selected so that only visible phenotypes are scored. Adequate training is necessary to achieve confidence in the scoring and a good level of reproducibility between experimenters. The test is designed so that no videotape recordings are needed; however, in particular experiments where subtle differences are expected a successive