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Paw reaching in rats: the staircase test

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1. DESCRIPTION

A variety of procedures have been employed to assess skilled hand and limb use in mice or rats, usually based on tasks in which the animals must reach through a slot[6], into tubes[18–21], from a tray[9,10,28], or from a conveyor belt[11] to obtain food. These experimental cages have been widely and effectively used to study the lateralisation of handedness[5,7,22,28], and switches in limb preference after different types of interventions[8–11,18–21,26–28]. In particular, several of these procedures have proved to be powerful for videotaped observation and description of the motor strategies adopted by the rats in executing reaching movements[6,25]. However, these classical procedures require than an observer must rate or count each reaching movement manually, which is labour intensive. Moreover, in order to investigate the function of the two forelimbs separately (e.g. on the sides ipsilateral and contralateral to a unilateral brain lesion), use of first one and then the other forelimb must be restricted, for example by application of a bracelet or injection of a local anaesthetic to immobilise one paw[12,17,23,28].

In order to circumvent the application of such constraints to the rat, an alternative strategy is to design the test apparatus so that the animal can only retrieve food from one location with one forepaw and from a second location with the other forepaw. Thus, Pisa[18–20], and Siegfried and Bures[21] placed food in tubes that were positioned up against the left and right walls of the test box, and into which the rat could only reach with the left and right paws, respectively. This procedure still requires performance to be continuously monitored and rated.

The present protocol uses a novel apparatus which has been designed to overcome these limitations. The 'staircase test' provides a new measure of paw use in rats in a task that is easily acquired, which allows the collection of objective quantitative data for both paws independently and from several animals tested simultaneously, and which has now been used as a reliable measure of impairment in several lesion paradigms[1–3,8,13,14,16].

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2. TYPE OF RESEARCH

A. The neuronal basis of limb control

The role of central nervous system structures and/or the control by aminergic neuronal systems of limb use were investigated in this task by measuring paw reaching after different kinds of lesions[1,8,13,14].

B. Study of the different parameters involved in limb use

This task permits the study of the influence of parameters such as motivation, previous training, etc., rather than just those concerned with the neuronal control of limb movement[14].

C. Recovery of function

1. *Study of developmental processes involved in spontaneous recovery after brain damage.* The demonstration that neonatal lesions spare paw reaching behaviour in the staircase test[3] indicates that it may be of use to evidence and to study the critical period during which the brain undergoes developmental changes which can influence the long-term effects of lesions.

2. *Spontaneous recovery after brain damage made in adulthood.* Since this task may be repeated, and animal performance remains stable after a long delay of training, the paw reaching test is appropriate for the study of recovery of function over time following partial lesioning of a given neural system.

3. *Drug-induced recovery after brain damage made in adulthood.* This task may be use to determine if performances in limb use may be improved by pretreating animals with drugs[1].

4. *Intracerebral neuronal transplantation.* The functional efficiency of intracerebral grafts has been evaluated by measuring the restoration of limb use disrupted by a previous lesion. These studies have been conducted for striatal grafts implanted in a previously lesioned striatum[13] and for dopamine-rich grafts implanted either into adult or neonatal brains, with a unilateral lesion of the dopaminergic mesotelencephalic system made during adulthood[1–3,8,13].

3. TIME REQUIRED

The animals should be well-handled and weighed before the outset of training. Training will then require that the animals are tested, in batches of 4–6 at a time, taking 10–15 min each day. The time required to train the animals for the task will initially take at least one week and may be repeated over several months according to the design of the experiment. Animals must be weighed and fed at the end of each test day in order to keep their body weights at approximately 90% of free feeding weight.

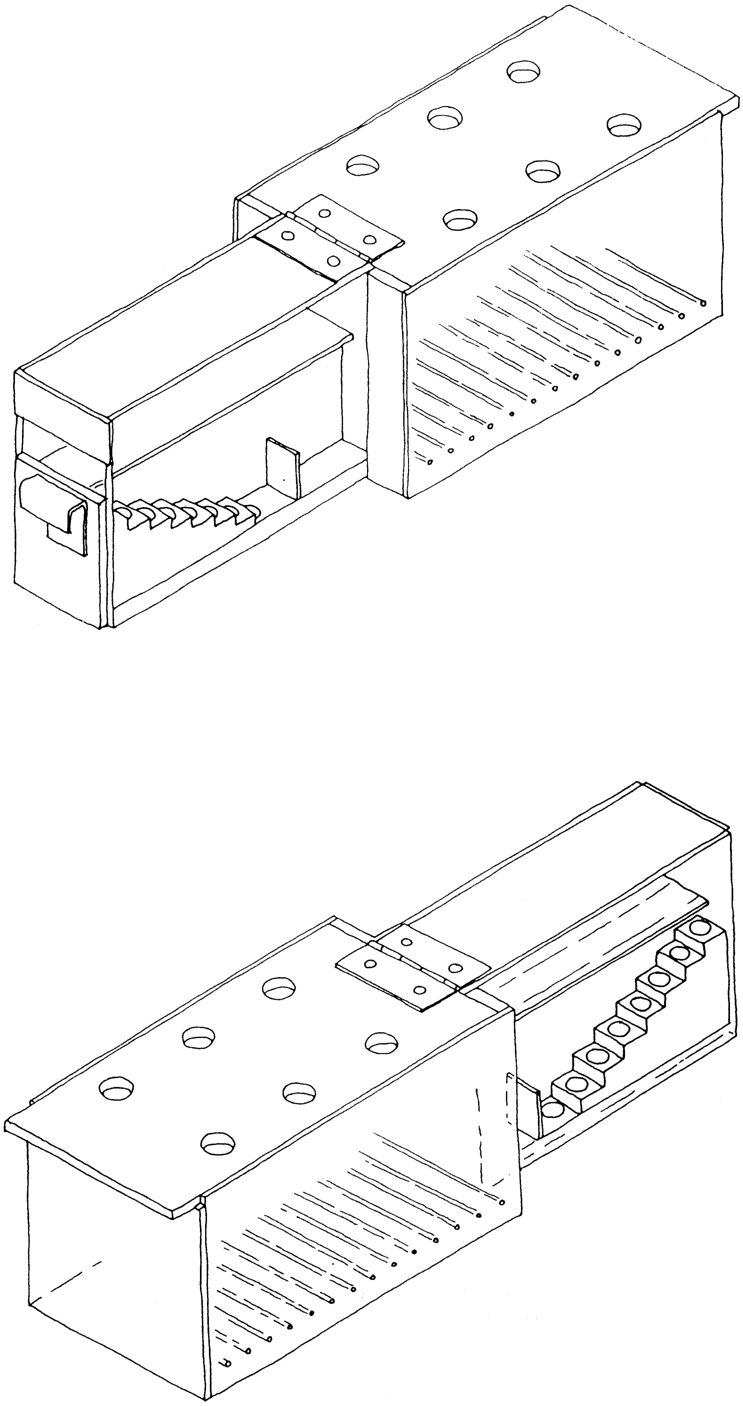

Fig. 1. An axonometric perspective: a front view (upper panel) and a back view (lower panel) of the staircase test.

4. MATERIALS

The apparatus (see Fig. 1), into which the rat is placed, consists of a clear Perspex chamber (203 mm long × 108 mm high × 103 mm wide) with a hinged lid. A narrower compartment (165 mm long × 108 mm high × 60 mm wide) with a central raised platform running along its length, creating a 19 mm wide trough on either side, is connected to the chamber. The narrowness of the side compartment prevents the rat from turning around, so that it can only use its left paw for reaching into the left trough, and its right paw for reaching into the right trough. The top surface of the platform is 35 mm wide, and hangs over the sides so that the rat cannot scrape food pellets up the side of the platform. A removable double staircase is inserted into the end of the box, sliding into the troughs on either side of the central platform. Each of the eight steps of the staircase contains a small 3 mm deep well, into which two food pellets are placed. Standard or dustless 45 mg chow or saccharin-flavoured pellets (obtained commercially from Charles River, Custom Biological or Bioserv) are all suitable. Sixteen pellets are placed on each side of the staircase. The highest step of the staircase is 13 mm below the central platform, and the bottom step is 64 mm below the platform. A hungry animal can collect pellets by reaching into the trough: the number of steps from which pellets have been removed provides an index of how far the rat can reach, and the number of pellets remaining at the end of the test indicates the rat's success in grasping and retrieving the pellets.

The animals are tested in a quiet room with a low level of background illumination.

5. DETAILED PROCEDURE

A. Housing and food-deprivation

1. One week before testing, animals are placed in individual cages. Single housing is preferable because it permits the weight of each animal to be monitored and maintained more accurately by corresponding adjustments of its daily food allowance. Rats are handled, weighed and placed on a food-deprivation schedule, being fed 8–12 g of lab chow at the end of the afternoon. Maintaining 90% of free-feeding weight during the period of testing has been found to provide a suitable level of deprivation for training in this test.

2. Three days after the beginning of food-deprivation, animals are familiarised to chow pellets, as rats can at first be neophobic towards novel foods. This is achieved by placing several pellets into the home cages twice per day over several days and verifying that they are eaten.

B. Training

Familiarisation sessions

The two sides are baited: two food pellets are placed into each well of the double staircase. The first day, animals are familiarised to the experimental cages by placing them into the test box for 15 to 30 min. On the second and third day of the experiment, the experimenter must help the animals localise the pellets placed in the wells during the 15-min sessions. For this purpose, some pellets are first distributed along the platform to attract the animals to the narrower compartment. The pellets are then presented with forceps in order to help the animals localise them. Additionally, six to eight pellets are placed into each well of the highest step of the staircase. The rat can easily reach these pellets with its tongue. If some animals fail to grasp the food, they might be trained more extensively (two sessions per day). Animals will begin to learn to reach food by the fourth day of the experiment.

Training sessions

Animals are placed in the test boxes once per day each day for 10 min. The staircase is removed at the end of each session, and the remaining pellets on each side are counted. To provide a simple comparison between groups, results may be expressed as the average number of pellets remaining in the staircase over the last five trials of the experiment.

1. *Standard conditions.* During this stage, both sides are baited. This phase determines the rat's reaching ability with each of its two forepaws. The 'preferred' paw is defined as the paw the rat uses to obtain the most pellets. Across a series of tests, animals will improve their performance: the number of remaining pellets in the staircase, on at least one side, will decrease. The animals can be considered to have learned the task when they reach asymptotic levels of performance, i.e. when performance is stable. This phase may be repeated in order to evaluate the effects of surgery (for example lesions) and recovery on paw reaching. Pre-and post-surgical performances in lesion and control groups are compared to evaluate the consequences of the surgery[1]. Testing may be continued (e.g. at monthly intervals) to investigate the stability of a deficit or a recovery of limb use across repeating testing[13].

2. *Forced paw use.* Just one side of the staircase accessible to the animals using a chosen paw is baited. This forces the animals to use only one paw for retrieving the food. It is useful to determine whether a unilateral lesion deficit may be attributable to the animal allocating a disproportionate attention to the unaffected side[2,14].

6. RESULTS

We have investigated the effect of a unilateral lesion of dopaminergic mesotelencephalic system on reaching ability and the efficacy of dopamine-rich grafts in alleviating a paw reaching deficit.

In all our experiments, control animals learn to reach the food from either side: few pellets (0–5 over a total of 16 pellets) remain on either side of the staircase. With extensive training, the performance of each forepaw becomes identical.

Observation of animals in the task indicates that normal rats will first attempt to retrieve pellets with their tongues. This is only successful for pellets in the highest food well, and normal rats continue to retrieve those pellets first before switching to using their paws to reach lower levels. The retrieval of pellets from the highest well can similarly be achieved by rats with profound impairments in limb use, and collection of these pellets provides a simple indication that lesioned rats maintain the basic motivation to respond[14]. By contrast, wells below this highest level are outside of tongue-reach.

The typical performance of a normal animal is characterised by rapid collection of the pellets from the next 2–4 steps within easy reach first on one side and then on the other within the first minute of the test. They will then spend several further minutes stretching towards the food wells at the limits of their reach and have generally given up attempts by the end of the 10-min test. The duration of the test session is therefore selected to provide the opportunity for each animal to retrieve the maximum number of pellets without time pressure. When pellets are well within reach, the normal animal grasps and retrieves the pellets without difficulty. However, the limits of reach, the animals may fail to grasp and drop the pellet or knock it down to a lower level beyond further contact. We routinely use the total number of pellets retrieved as a measure of successful reaches. Whereas a more detailed analysis of the numbers of pellets left on each step of the staircase, dropped or displaced might be expected to indicate additional clumsiness in responding[14], we have not found that this additional analysis provides useful information in practice.

A number of lesions produce marked deficits in the staircase test of paw reaching, of which we have studied the consequences of nigrostriatal damage in most detail. Rats show little or no deficit in reaching with the paw ipsilateral to the lesion side, whereas they are unable to collect pellets with their contralateral paw: between 10 to 16 pellets remain on one side of the staircase. The fact that even the two pellets in the highest well are not collected in rats with the most extensive dopamine depletion indicates that the deficit is not solely motor. Motivation is clearly intact in view of their performance on the intact side; rather these animals fail to even attempt to retreive pellets from the contralateral side, suggesting that the unilateral lesion deficit involves at least in part a motor neglect of contralateral space. This contralateral deficit is stable across several months of testing[13]. Even when forced to use their non-preferred paw (contralateral to the lesioned side), lesioned animals do not improve in their reaching performance[2]. The extent of this deficit seems to depend on previous training: deficit in paw reaching with the contralateral paw is weaker for animals which have been trained for paw reaching before the lesion procedure (comparison of results obtained from refs. 1 and 2). Lesions carried out early in infancy produce a less marked deficit in the use of the contralateral paw than is seen when lesioning occurs at a later stage of development[3]. It has proved difficult to alleviate deficits in paw reaching by dopamine neurones implanted in the dopamine depleted neostriatum of adult rats[1,13], even when the dopamine grafted neurones are stimulated by pre-treatment with amphetamine[2].

7. DISCUSSION

The staircase test is a simple and efficient test to assess limb preference in rats, and in contrast to other tests the task does not require detailed observation of the animal's behaviour for basic data collection. Consequently, the organisation of behavioural sequences based on videotaped recording which lead to paw reaching (e.g. component movements of reaching, sensory control and guidance of reaching, speed and reaction times of movement) which have been analysed in detail in other tests[2,11,23,25] have not yet been studied in this test.

Furthermore, handedness has already been studied in rodents, particularly mice, in order to: study brain lateralisation[4,24], determine the genetic components of handedness[5], and examine the possible association between paw preference and immune reactions[15]. By contrast, observations of normal animals in the standard version of the staircase test revel few detectable asymmetries. Since the degree of lateralisation of limb use in control animals is weak when compared to the strong asymmetry reported using other test procedures[28], the staircase test may not be sufficiently sensitive to study spontaneous (as opposed to lesion-induced) limb preferences.

However, to offset these weaknesses, the staircase test has several advantages:

- The test scores are simply counted at the end of each test session rather than requiring constant monitoring, rating or judgement by the experimenter.

- The test provides separate measures of the reaching ability of the two forepaws independently.

- It yields stable and reliable data with repeated measures across testing.

- It offers the possibility to constrain rats to use of only one forepaw at a time with no additional restraint.

The staircase test therefore provides a simple and efficient screen of motor deficits following central lesions and subsequent recovery, whether due to spontaneous processes of reorganisation or to explicit experimental treatments.

7.1. Trouble shooting

A. If animals do not learn the task

i. *Increase food-deprivation;*

ii. *Verify that animals are not staying in the departure compartment;*

iii. *Ensure that rats eat the pellets;*

iv. *Expand the familiarisation phase by exposing the animals more frequently to the test boxes and by placing pellets in the home cage more often.*

B. Animals might develop strategies to overcome their deficits

An animal with a lesion of the dopaminergic system made on its left side exhibits a contralateral deficit in limb use, i.e. it does not grasp pellets located in the right trough. Animals weighing less than 250 g can turn around in the compartment and use the left paw to reach the pellets. The dimensions of the cross-section of staircase apparatus should be reduced as appropriate to overcome this problem.

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D. Nora Abrous and Stephen B. Dunnett, Paw reaching in rats: the staircase test, Neurosci. Prot., 94-010-03

QUICK PROCEDURE

Housing and handling

A. House rats individually if possible. Weigh.

B. Establish food-deprivation schedule to maintain 90% of free feeding weight.

Familiarisation session

C. Rats are exposed to the staircase boxes for 15 to 30 min per day until they begin to reach pellets located in the staircase.

Testing procedure

D. Place rats in the staircase boxes for 10 min per day, with both sides baited.
At the end of the session, remove the staircase and count the number of remaining pellets.
Animals are considered to have acquired the task when a stable level of performance is maintained.
During this phase, the non- preferred paw is defined as the paw that the animal uses less often to obtain pellets.

E. Rats may be constrained to use their 'non-preferred paw' by only baiting one side of the box.
Assess whether preferential attention to one side affects performance by baiting each of the two sides on alternate days.