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Lab Anim 2001 35: 340
DOI: 10.1258/0023677011911930

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What is This?
The toe-spreading reflex of the rabbit revisited—functional evaluation of complete peroneal nerve lesions

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Summary
Although a variety of electrophysiological and morphological tests are available for studying nerve regeneration in animals, these endpoints do not necessarily correlate with the return of muscle function. Recent efforts have focused on the assessment of function as the endpoint of nerve regeneration. One of the best known of these tests is the sciatic function index in rats. For rabbits, the toe-spreading reflex has been suggested as a valuable index of peroneal function. We examined the reliability and sensitivity of the toe-spreading reflex in a study of nerve regeneration of the peroneal nerve in 10 New Zealand White rabbits. Eleven weeks after the transection and immediate suturing of the peroneal nerve in both hind legs (at two slightly different sites), a toe-spreading reflex could always be elicited on that side where the level of the severed nerve was closer to the dependent muscles. Also on this hind leg the muscle weight of the peroneal target muscles was significantly higher ($P < 0.031$) than on the contralateral side, which corresponds well to the results of the toe-spreading reflex.

The toe-spreading reflex is an excellent and sensitive indicator of the onset of motor recovery in the peroneal nerve-dependent muscles of rabbits. Even small differences in the localization of lesions in both hind legs can be differentiated with this test.

Keywords Peroneal nerve; rabbit; nerve regeneration; functional evaluation; toe-spreading reflex

Studies of nerve regeneration in animals make use of a variety of electrophysiological and morphological tests, but these endpoints do not necessarily correlate with the return of muscle function. Recent effort has focused on the assessment of function as the endpoint of nerve regeneration (Hare et al. 1992).

In the clinical situation, nerve recovery can be assessed by the return of functional abilities such as pinch and grip strength. Unfortunately, counterparts to such functional tests are unavailable in experimental research with animals. Consequently, other valid functional tests, indicating the definite return of voluntary or reflex movements in muscles, have been sought. De Medinaceli et al. (1962) introduced the sciatic function index (SFI). This is an index of the functional condition (recovery) of the rat sciatic nerve based on measurements from walking tracks. This has since been further subdivided into a tibial function index (TFI) and a peroneal function index (PFI) (Bain et al. 1989). These walking track analyses have come into wide use. They are said to be the best experimental measure of...
muscle function in rats and may most closely approximate the clinical situation.

Although the rat is the experimental model of choice for many peripheral nerve investigators (Mackinnon 1986), important findings should be reconfirmed with similar experiments using more highly developed animals, prior to their extrapolation to the clinical situation. In addition, there are also experimental models that demand a more complex nerve regeneration in a larger animal, more closely imitating the human situation. When for one of these reasons as in our experiment, a rabbit model is selected, the tibial or the peroneal nerve may provide a more reliable and sensitive means of studying nerve regeneration than the sciatic nerve. This has already been shown in the rat model (Hare et al. 1992). Given the choice, the peroneal nerve is undoubtedly to be preferred over the tibial nerve, as the muscular impairment is significantly more discrete and the well-being of the animals is less compromised. A few days after the complete section of the peroneal nerve, a rabbit can walk on a level surface in a manner not immediately distinguishable from normal. What is an evolutionary advantage for the animals, becomes a challenge for the researcher needing to measure muscle function. Upon closer observation, there is a tendency for the hock joint to be fixed in an everted position, and for the paw to rest more on the phalanges. In addition, due to the lost ability of toe-spreading, the toes are adducted (and the feet impose therefore a little smaller). These features do not have a marked influence on the stance and gait, except for some abduction of the foot when sitting and some dragging of the foot when hopping. Although the dropfoot is marked immediately after the interruption of the nerve, it diminishes or disappears often long before the reinnervation of the muscles has taken place (Gutmann et al. 1942). This is due to the sitting posture of the rabbits, which fixes the foot in a bent position. Comparative walking analyses have shown that rabbits with or without lesions of the peroneal nerve can hop equally quickly.

The discreteness of the symptoms of peroneal nerve injury means that observations of the gait alone are insufficient to estimate the exact onset of motor recovery. A more reliable estimate of the onset and progress of recovery is the restriction of observations to the muscle group innervated specifically by the peroneal nerve. Movements which may be elicited by reflex are advantageous as indicators of the onset of motor function, as errors and artefacts due to trick movements are avoided. One of these reflex movements in rabbits was mentioned casually by Gutmann (1942): he described the spreading of three toes of the hind limbs after peroneal nerve lesions and recovery. These muscles may be induced to contract reflexively, as Langley (1917) first observed in cats, by holding the animals by the loose skin of the back and then suddenly lowering them in the air without letting them touch a surface. At this time, the animals spread their toes reflexively in an attempt to enlarge the surface area of their feet for a safer landing. The muscles of the rabbit involved in this reflex are the peroneal II, III and IV muscles innervated by the peroneal nerve (Craigie 1969). These peroneal muscles spread the 2nd, the 3rd and the 4th toes, and thus constitute a reflective ‘peroneal nerve function index’ (PFI) for rabbits (Gutmann 1942).

During regeneration of the peroneal nerve the first response is a mere flicker of the 4th toe. The spreading increases in amount from day to day, gradually involving the second and the third toes. Gutmann (1942) created an arbitrary 4-step scale (the ‘spreading index’, degree I–IV; Table 1) to describe this gradual return of peroneal nerve function. We hypothesized that this index might be applicable to the monitoring of recovery in a single animal, and to express differences in the final degree of recovery attained following different lesions.

Therefore, in a study of the recovery of the peroneal nerve in rabbits, the reliability and sensitivity of the toe-spreading reflex was evaluated as a sign of functional recovery alongside concurrent muscle weights.

Material and methods

Animal model

Ten adult, female New Zealand White rabbits ranging in weight from 3.6 kg to
4.3 kg (mean 3.9 kg) and with a conventional microbiological status were chosen for the experiments. They were maintained in a central animal care facility under conventional optimal hygienic conditions and controlled environmental conditions. They were housed in groups of five in structured light grey plastic cages (Tecniplast, 6000 cm$^2$ each) set five in a row. The side walls of the cages contained sliding doors that were kept open. The cages were further equipped with perforated plastic floors and a hiding place for the animals to retreat to. The animals received rabbit chow (Kliba), fresh vegetables in limited amounts and water ad libitum, along with pieces of wood for them to gnaw on. Environmental conditions in the room were: temperature 21°C ± 0.5°C, relative humidity 50–70% and air exchange rate of 10 room volumes/h. The light/dark cycle was 12/12 h with artificial light from 06:30 to 18:30 h in addition to normal daylight. During the day phase a radio music programme was played in the animal room. All surgical procedures were completed in accordance with the National Institute of Health guidelines and Swiss animal protection laws (permit number 183/99) for the use of laboratory animals for research purposes. The animals used were part of a comprehensive experimental study on conditioning phenomena and electrophysiological studies.

### Table 1 Peroneal function index (PFI)—Toe-spreading index (Gutmann 1942)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Clinical symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree I</td>
<td>Just visible spreading of the 4th toe alone (also 2nd and 3rd)</td>
</tr>
<tr>
<td>Degree II</td>
<td>Slight spreading of all three toes</td>
</tr>
<tr>
<td>Degree III</td>
<td>Spreading of all three toes less forceful than normal</td>
</tr>
<tr>
<td>Degree IV</td>
<td>Full spreading of all three toes equal to normal</td>
</tr>
</tbody>
</table>

#### Surgical procedure

The rabbits were given a premedication with ketamine 65 mg/kg (Gräub), and xylazine 4 mg/kg i.m. (Bayer) before oral intubation. Anaesthesia was induced with propofol 4 mg/kg i.v. (Abbott) and maintained with isoflurane 1–4% (Abbott), O$_2$ and N$_2$O whilst the animals were breathing spontaneously. For the peri- and postoperative analgesia buprenorphine 0.01–0.05 mg/kg i.m. (Reckitt & Colman) was given. Likewise a prophylactic antibiotic of sulfadoxin/trimethoprim solution 24%, 20/4 mg/kg i.m. (Hoechst-Roussel) was administered.

The central part of the peroneal nerve was exposed through a muscle-sparing incision along the sciatic vein between the semi-tendinosus and the biceps muscle (Cristina Schmitz & Beer 2001). The two muscles were bluntly spread to expose the peroneal nerve.

#### Experimental design

The experimental design was:

1. Conditioning crush lesion on the peroneal nerve: on the left side at the border of the semi-membranous and adductor magnus muscle, on the right side 10 mm distally.
2. Test lesion (complete transection and suturing) 4 weeks later: on the right side at the border of the semi-membranous and adductor magnus muscle, 10 mm proximal to the conditioning lesion, on the left side 10 mm distally.
3. Toe-spreading reflex testing until the onset of motor recovery on at least one hind leg (11 weeks after transection).
Prior to crushing, the integrity of all peroneal nerves and target muscles was checked by compound muscle action potential (cMAP) recording. All cMAP values were normal, confirming an initial, normal toe-spreading reflex. Then, in both legs the conditioning crush lesion was produced with a serrated haemostat (Micro-Mosquito U10/32112, Ullrich) for 45 s (Beer et al. 2001). The effectiveness of crushing was evaluated with a control EMG (zero line).

Four weeks later, the nerves were transected, either 10 mm distal (left side) or 10 mm proximal (right side, control) to the crush site, i.e. on the left side transection occurred closer to the dependent muscles. The lesions were immediately sutured epineurally with four stitches of 10/0 nylon and additionally glued with 0.3 ml of fibrine solution (Immuno). Coaptation of the left lesion was carried out on the freshly regenerating nerves, whereas on the right side, coaptation was in the region of the intact nerve.

Starting 9 weeks after the second operation, which was the earliest that the onset of motor recovery could be anticipated, the rabbits were regularly tested for the return of the toe-spreading reflex. After motor recovery on at least one side and a toe-spreading reflex with an obvious spreading index of degree III, the nerves were exposed once more (11 weeks postoperatively). Prior to this operation, photographs (Fig 2) were taken of both hind legs during the toe-spreading reflex test, scanned and compared to the initial photographs of the unimpaired toe-spreading reflex. To avoid bias in the results due to the scanning procedure (enlargement or reduction in the size of the photograph) the photographs were superimposed and the differences in toe-spreading between the two sides (left vs right) were indicated as percentages (automatrical calculation, Photoshop, Image Access) of the normal (Fig 2c).

Immediately after euthanasia with pentobarbital 162 mg/kg i.v. (Veterinaria) the four peroneal muscles were removed from each hind leg. As the single muscle is very delicate the muscles were not separated. They were weighed and the difference between left and right hind legs was compared to the results of the toe-spreading reflex.

**Statistical analysis**

Statistical analysis was performed using StatView 5.0.1. (SAS Institute Inc., Cary, NC). Comparison of the right and left peroneal nerve was carried out with the Wilcoxon signed rank test. \( P \) values \( \leq 0.05 \) were considered to be significant (2-tailed).

**Results**

Eleven weeks after the second operation (complete severance of the peroneal nerve), a toe-spreading reflex degree III could be demonstrated clearly and easily in all 10 rabbits on the left side, whereas on the right side it was still completely lacking. The scanned photographs provided a convenient method for quantifying the spreading index of Gutmann (1942) by calculating the percentage of impairment of toe-spread in comparison to the normal (Fig 2c).

Antemortem, all peroneal nerve-dependent muscles on both sides showed the characteristic yellowish-brown colour as a typical sign of atrophy. The difference in the mean weight of the peroneal muscles on the left (2.36 g) and the right side (1.92 g) was statistically significant \( (P = 0.031; \text{Table 2}) \).

**Discussion**

Eleven weeks after severing the peroneal nerve, all the animals had a toe-spreading reflex G III on the left side. This reflex could be elicited without difficulty in all the animals. The results showed a clear, significant difference in the toe-spreading reflex (PFI) between the left and right hind legs, although the difference in the localization of the test lesion was only 10 mm. These results are strong indicators of the sensitivity of such a test. The same applies to the weight of the associated peroneal muscles, where a significant difference could be found between both hind legs, despite the short temporal difference for nerve regeneration.

These findings contrast with the difficulties experienced in the evaluation of the PFI in rats using walking track analyses. One of the difficulties is that several walks are often required in order to obtain clear print marks.
of both feet. Some prints cannot be analysed owing to smearing of the print, dragging the tail across the print, or contamination with front prints (Bain et al. 1989). Furthermore, prior to any surgery, the rats must be trained to walk in the tracking corridor. Another difference between the PFI of the two species is that the PFI of rabbits involves a simple reflex movement which can be photographed, computed and measured automatically by the computer programs, whereas the PFI in rats requires the combined

![Fig 2 Examples of the toe-spreading reflex of the rabbit. (a) Complete peroneal lesion on both hind legs. (b) Complete peroneal lesion of one (left) hind leg. (c) Unidimensional measurement of the amount of toe-spreading in a paralysed rabbit’s foot in contrast to normal (= 100%; = Fig 2e). Two photographs (pre-lesional and post-lesional) are scanned into a computerized drawing program (Photoshop) and superimposed. Vertical lines are drawn along the most prominent parts of the hind feet and the width is automatically measured by the program and the percentage is indicated. (d) Toe-spreading reflex G III of one (left) hind leg. (e) Toe-spreading reflex G IV on both hind legs (preoperatively).]
analysis of four parameters (distance to the opposite foot, print length, toe spread and the intermediate toe spread) using a mathematical formula, the result of which indicates the degree of function or regeneration. In general, the indicators of peroneal nerve lesions in rats are more discrete than in rabbits. In a study similar to ours, no significant differences in PFI could be found in rats following left/right differences in peroneal nerve lesions (Bain et al. 1989). Although the print length was shortened, the animals showed only a slightly decreased toe spread with the distance between the intermediary toes remaining relatively unaffected. However, it is known that determining the toe spread in rats using walking track analysis alone can be difficult (De Medinaceli et al. 1962). Manual measurement of toe spread in rats has been shown to be a more reliable alternative. The rat is held suspended by the skin at the nape of the neck, positioned vertically as if it were standing on its hind limbs only, and the toe spread measured. This factor has even been used as a sole measurement of sciatic function in rats (Bain et al. 1989), however it should be reserved to isolated peroneal lesions.

In summary, the toe-spreading reflex of the rabbit is an easy and reliable test requiring neither prior training of the animals nor walking track analyses. The rabbits are simply held at the nape of the neck and suddenly lowered. The test can be repeated as often as necessary. It is an excellent indicator for the onset of motor recovery in the peroneal nerve-dependent muscles and allows small differences in the sites of lesions in both hind legs to be detected. The PFI test offers the peripheral nerve investigator a non-invasive quantitative assessment of peroneal function in the rabbit with selective peroneal nerve injury.

Finally, the peroneal nerve of the rabbit is a rewarding model of peripheral nerve regeneration for situations where a larger animal model than the rat is required. The model has the further advantage that the lesions do not grossly interfere with the welfare of the animal.

References
Gutmann E, Gutmann L, Medawar PB, Young JZ (1942) The rate of regeneration of the nerve. Journal of Experimental Biology 19, 14–44
Langley JN (1917) Observations on denervated and on regenerating muscle. Journal of Physiology 51, 377–95

Table 2 Weight (g) of the peroneal muscle group on the left and right hind legs of the rabbits

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
<th>R9</th>
<th>R10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>2.03</td>
<td>3.19</td>
<td>1.60</td>
<td>2.07</td>
<td>1.72</td>
<td>3.20</td>
<td>2.24</td>
<td>2.86</td>
<td>2.38</td>
<td>2.33</td>
</tr>
<tr>
<td>Right</td>
<td>2.05</td>
<td>1.38</td>
<td>1.38</td>
<td>2.05</td>
<td>1.19</td>
<td>2.36</td>
<td>2.00</td>
<td>2.61</td>
<td>1.95</td>
<td>2.25</td>
</tr>
</tbody>
</table>

R1–R10 = number of the rabbit