A technique for the surgical removal of a kidney from individuals of a feral population of small rodents

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Received March 27, 1984

PAVONE, L. V., and R. BOONSTRA. 1984. A technique for the surgical removal of a kidney from individuals of a feral population of small rodents. *Can. J. Zool.* **62**: 2146–2149.

A technique for the surgical removal of a kidney from small rodents is presented. It involves making two incisions, tying off the ureter and the renal blood vessels, removing the kidney, and stitching the incisions. Under laboratory conditions virtually all animals recuperated. A field test resulted in a 78% recovery rate for treated animals compared with their controls. The technique is simple, inexpensive, and quick making it a potentially useful tool in many areas including paternity studies and gene flow studies.

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On trouvera ici la description d'une technique d'ablation chirurgicale du rein chez de petits rongeurs, technique qui consiste à pratiquer deux incisions, à ligaturer l'uretère et les vaisseaux rénaux, enlever le rein et recoudre les incisions. En laboratoire, presque tous les animaux se remettent bien de l'opération. En nature, la récupération des animaux opérés a révélé un taux de réussite de 78% par rapport aux animaux témoins. La technique s'avère simple, peu coûteuse et rapide; elle sera donc utile dans plusieurs types de recherches, par exemple dans des études de paternité et des études sur la circulation des gènes.

[Traduit par le journal]

Introduction

Since Lewontin and Hubby (1966) applied electrophoretic techniques to investigate molecular variation in natural populations of *Drosophila pseudoobscura*, many biologists have used the same techniques to monitor allelic frequencies of structural and metabolic proteins in rodents (e.g., Russell and Semeonoff 1967; Semeonoff and Robertson 1968; Tamarin and Krebs 1969; Gaines and Krebs 1971). Still others have used them to estimate genic heterozygosity (Garten 1976; Massey 1977).

Surveys which were done in association with live-trapping studies did not allow the sacrificing of animals. These studies tended to rely on plasma and haemolysate, or saliva for their protein sources. It is not surprising that these studies examined only a few proteins.

Biologists who sacrificed animals examined a larger number of proteins (Selander *et al.* 1971; Garten 1976; Massey 1977; Bowen and Yang 1978; Schmitt 1978). The results of these larger surveys make it clear that the kidneys are a source for many enzymes which are readily scored on gel electrophoresis.

This paper describes a technique for the removal of a kidney from individuals of feral populations of small rodents. The technique is simple to learn and to use, and it can be efficiently used by a single worker on large populations.

M. C. Baker (1981) developed a biopsy using the muscle tissue of birds, and Baker (1983) has since applied the technique to mouse populations.

Materials and methods

Preparation for surgery

Animals are anesthetized with 0.075 mg Ketamine HCl/g of animal (source: "Ketaset," Rogar/STB division of BTI Products Inc., London, Ont., Canada), and 0.015 mg sodium pentobarbital/g of animal (source: Somnotol, MTC pharmaceuticals, Hamilton, Ont.). The drugs are administered subcutaneously in opposite sides of the abdomen. The amounts of Ketamine HCl and sodium pentobarbital used will vary from specimen to specimen and it may require some practice

before a worker can successfully administer the drug. This initial step seems to have been the most critical in the entire procedure. The mouse will be immobilized within minutes. If after 10 min the mouse is still sensitive to a pinch of its tail, more of the drugs may be necessary.

Surgery

A small patch of skin above the right kidney is shaved and disinfected. Using a pair of alcohol-sterilized, rat-toothed forceps and scissors, we made two 1-cm incisions: one on the skin and the other through the body cavity parallel to the second- or third-last rib thereby exposing the chest cavity. The right kidney is directly below these ribs (Fig. 1). A pair of retractors are used to keep the incision open (Fig. 2). One end of a surgical thread (gut or silk, medium gauge or thinner) is tied (No. 1, Fig. 2) to a pair of wide-tipped forceps and a single loop of thread is placed over the forceps' tip (No. 2, Fig. 2). This arrangement (Fig. 3) will allow a worker to slip a loop (No. 1, Fig. 3) over the kidney once the kidney is pinched with the forceps. Failure to use this or a similar method will require an additional worker to tie off the ureter and renal blood vessels. A second insurance knot should be made to secure the initial knot (Fig. 4).

The kidney is then cut off (Fig. 5) above the surgical thread. The ureter and renal blood vessels should be secure with minimal blood loss. If, at any time, a substantial amount of blood is being lost, simply apply pressure to the injured area with clean, cotton-tipped applicators. A few minutes of pressure will eliminate seemingly severe blood loss.

Finally, the two incisions are sewn up (Fig. 6). The mouse is replaced in its cage and covered with cotton to minimize heat loss. Antibiotics are not usually required since the immune system seems to be very well developed in feral populations. We estimate that this procedure can be successfully performed on four animals per hour.

Effects of surgical procedure on survival

In the laboratory

A total of 70 laboratory-reared meadow voles (*Microtus pennsylvanicus*) of various ages and sexual conditions had their right kidney surgically removed. Following surgery, voles were individually housed in opaque polypropylene cages with water and laboratory rat and rabbit chows provided *ad libitum*. Animals were checked the day following surgery and every few days until the incision had healed (10–14 days).

In the field

A live-trapped population of voles at Toronto's Pearson Inter-

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FIGS. 1-6. A step by step example of the procedure. Fig. 1. Initial incisions through the skin and body cavity. Fig. 2. Configuration of the surgical thread on the forceps. Fig. 3. Slipping the thread over the kidney. Fig. 4. Tying off the ureter and renal blood vessels. Fig. 5. Removing the kidney. Fig. 6. Closing the incisions.

TABLE 1. Summary statistics of the kidney-removed and control populations of meadow voles

	<i>N</i>	Female voles	Male voles	Average weight (g)	No. of voles surviving	No. of voles on perimeter	Proportion of surviving voles caught on perimeter
Kidney removed	23	15 (65%)	8 (35%)	41	15 (65%)	12 (52%)	0.47
Control	23	15 (65%)	8 (35%)	42	20 (87%)	14 (60%)	0.55
Total	46	30	16				
Proportion of voles surviving the surgery = $(15+3)/23 = 18/23 = 0.78$							
Proportion of unaccounted voles trapped on the perimeter = $5/5 = 1.00$							

national Airport was used to test the effects of surgery on the survival of feral individuals. Voles had already been ear-tagged and toe clipped so that any immediate deleterious effects from these procedures would have already taken place.

During each trapping session voles were paired with respect to weight, sex, and sexual condition. One vole from each pair was randomly selected for kidney removal while its partner served as the control. Kidney removals were performed within hours of trapping. Both members of a pair were returned to the area within 2 h of the surgery, once the treated vole regained full mobility. A total of 23 pairs of voles were used over a 2-day period. Survival of these voles was estimated by subsequent semiweekly trapping up to 3 weeks after their release. This experiment was conducted from October 16 to November 5, 1983, a period of low temperatures (average maximum, 10.4°C; average minimum, 1.0°C).

Results

Laboratory

Of the 70 voles used in the laboratory experiment, only 4 died from the procedure. Three died from a drug overdose and one died overnight when it tore its stitches. In effect, the surgical procedure is virtually 100% successful. It was difficult to find any trace of the surgery after a period of 10–14 days, except for the bare spot where the fur had been shaved.

Field

Table 1 summarizes data on the two groups of voles used in this experiment. Two weeks after the surgery a total of 15 (65%) animals from the kidney-removal group and 20 (87%) from the control group were known to be alive. The deaths in the control group were not treatment related and we assumed that the natural death rate was constant in the two groups of voles. If we were to eliminate deaths owing to natural causes from the kidney-removal group's results, only 5 (22%) animals, all trapped along the perimeter, could not be accounted for, and we assume this may be the additional mortality caused by the operation.

Discussion

We conducted our field trial in a manner calculated to increase the probability of death following the operation; i.e., we performed the trial during cold weather and we kept the postoperative time to a minimum. Nevertheless, the operation was responsible for only 22% mortality. Finally, all animals which we classified as missing and presumed dead had been trapped on the perimeter of the grid (Table 1). The results of continuous live trapping are, in part, influenced by edge effect. Animals which tend to be found near or on the edge of the trapping grid are more difficult to trap since fewer traps are available to

them. Although this factor was generally controlled between the two groups, analysis of recapture data revealed that all unaccounted voles were originally trapped on the perimeter. It is possible that some of these animals had moved away from the area.

The risks of field operations can undoubtedly be mitigated. Animals could be brought into the laboratory, operated on, and then released after a 2- to 3-day postoperative recuperation period. Or the operations could be performed in the field but the animals held for recuperation in portable cages for 2–3 days. In either case, increasing the postoperative recuperation time should increase the success of the operation as was demonstrated by the virtual 100% recovery of animals in the laboratory. However, removal of the animals from the population for this length of time may also disturb the social structure and the dynamics of the population.

Although we have described and tested only the surgical removal of a kidney, the technique could be adapted to removing a small portion of the liver from the same incision. The difference between the two methods is that the liver cannot be tied off as the ureter and renal blood vessels have been. However, using cotton-tipped applicators pressure could be applied to the area until the bleeding stops.

Application of the kidney-removal technique may become useful in various studies. The most obvious is in studies dealing with changes in allelic frequencies or heterozygosity and the dynamics of a feral population. This technique will allow a biologist to increase the number of proteins monitored without sacrificing individuals. It may also be of use in studies attempting to untangle paternities (e.g., Foltz 1981). Another application is in the examination of gene flow in a free-living population (A. E. M. Baker 1981).

Finally, the inheritance pattern of many of the enzyme systems concentrated in the kidneys and currently being studied in natural rodent populations have not been thoroughly untangled. Because of the inability of the researcher to identify the partners of a possible mating prior to breeding, loci with rare alleles are difficult to deal with. For example, if the frequency of a heterozygote in a diallele system is 10%, it would take 100 randomly selected breeding pairs to expect only one mating of heterozygotes. Using the kidney-removal technique, individuals could be selected for mating based on their enzymatic spectrum.

The kidney-removal technique can thus be applied successfully under certain conditions. The success one may expect depends on the species used, general health of the animals, expertise of the worker, and the environmental conditions to which the animals are subjected.

Acknowledgements

We wish to thank V. L. Primomo, J. Chin, B. Bank, C. Ewing, and Dr. D. Chute for their assistance with various aspects of the surgical technique. Drs. J. Bendell, L. C. Cwynar, and M. Gates made many valuable comments on an earlier draft. The comments of two anonymous reviewers greatly improved the quality of this paper. This research was funded by a Natural Sciences and Engineering Research Council of Canada grant to R.B.

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