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Research Articles

A new marker technique in cineradiography for the recording of movements in small vertebrates – Application to the study of jaw movements in soricids (Insectivora)

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Summary. Dental parapulpar pins (TMS® Link Series) are reliable markers in cineradiography for the recording of movements in small vertebrates. The application of the pins to an analysis of mandibular movements of soricids allows a reconstruction of the complex movement pattern of both jaws during mastication.
Key words. Marker pins; cineradiography; movement recording; mastication; soricids; shrews.

Cinefluoroscopy often uses the natural landmarks of the skeleton to detect motions, e.g., those of the jaws during mastication [1]. This may, however, be inadequate for the analysis of the complex jaw movements of small verte-

brates. The introduction of lead markers or amalgam in sticklebacks[2], rats[3], rabbits[4] and bats[5], and screws in tenrecs[6] has led to better results.

The complex mandibular movements of soricids are difficult to analyze cinematographically[7,8]. The principal reason is the small size of shrews. The unique patterns of jaw movements[9] requires the recording of mandibular rotation and not just translational movements.

The fundamental problem is that an element moving in space, e.g., the lower jaw, appears on a film with its coordinates only in a single plane. Usually, three points per rigid moving element serve as markers in order to reconstruct motions in the third dimension. If the plane of the object rotates relative to the projection plane, additional markers out of plane of the three marks have to characterize negative or positive rotation. The techniques of solid geometry then permit a reconstruction. However, we cannot detect translations of the body perpendicular to the projection plane. In that case, a different angle of projection has to be chosen. This paper reports on the technique of using self-threading pins as reliable markers during cineradiography for the recording of small movements. An application to the jaw movements of shrews provides a three-dimensional reconstruction of their chewing pattern.

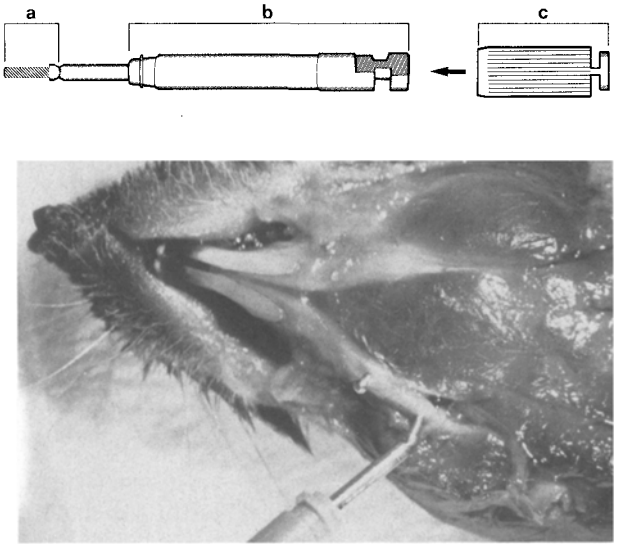

Figure 1. Minuta® single-shear pin (above), *a* pin, length 2 mm, *b* plastic shank, *c* hand driver. Below: implanted pin on the right lower jaw (left), bending of the pin (right) in *Blarina brevicauda*.

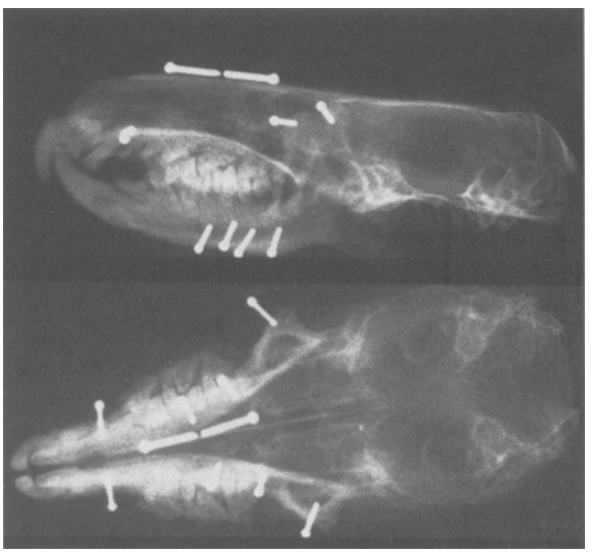

Figure 2. Lateral and dorsoventral radiographs of the head of *Crocidura flavescens* with implanted pins, displaced at an angle of 90°.

Materials and techniques

The dental TMS® Link Series self-shearing gold plated stainless steel pins (Minuta® single-shear, length 2 mm, shaft diameter 0.35 mm, and Minikin® single shear, length 3 mm, shaft diameter 0.35 mm, see fig. 1) were implanted on or mounted surgically on the skulls of several shrew species. *Suncus murinus, Crocidura flavescens* (Soricidae, Crocidurinae) weighed 40–80 g, and *Blarina brevicauda* (Soricinae) weighed 20–26 g. The pins were implanted under general halothane anesthesia (for details of surgery and the behavior of the shrews during anesthesia see Dantuma and Dötsch[10]). The TMS® Link Series pins consist of a self-threading pin (fig. 1,a) linked to a resilient plastic shank (b). This shank aids in the alignment of the pin to the drilled channel for smooth and safe insertion. The shank fits directly into a universal hand driver (c).

After removal of the periosteum, a spot was marked in the bone with a round bur No. 1/4. Then a channel was drilled in one smooth pass with a suitable Kodex® drill. The insertion depth of the pin could be determined during this procedure. Chips of bone were removed by washing out with isotonic solution. (One has to be careful of blood vessels near the rotating bur. A small amount of bleeding may occur, but it stops immediately when the pin is installed). The insertion was easily made with the hand driver (fig. 1). The plastic shank was then carefully bent manually, using a pair of pincers to support the screw-head of the pin in order to prevent damage of thin bone. The pin was thus broken at a point just behind its screw-head.

The X-ray photograph in figure 2 illustrates the position of the pins in both lateral and dorsoventral views. Two pins were implanted in the compact bone of each mandible, one in the anterior, the other in the posterior part (fig. 3, left jaw, pins with screw-head No. 11, resp. 13). It is important to implant the pins in different directions. In lateral cineradiographic films this permits unequivocal differentiation between pins inserted into similar positions on the right and left mandibles. A third pin was inserted at the tip of the coronoid process (screw-head No. 9). Pins were also installed bilaterally on the

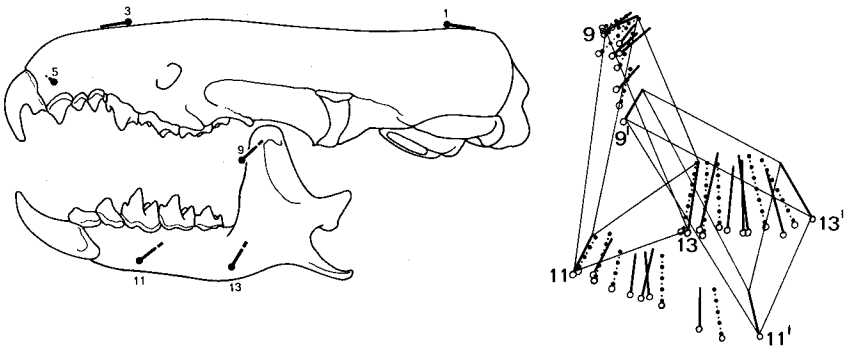

Figure 3. Lateral view of the skull of *C. flavescens* with pins; connection between points 1 and 3 is the reference line used to reconstruct a model of the movement pattern of one chewing cycle (duration is 240 ms, changes from frame to frame are 20 ms), left jaw. Explanation in text.

skull into a portion dorsal of the unicuspid teeth (in fig. 3 left side, No. 5). As the weakness of the compact bone roofing the braincase did not permit implantation of pins, they were instead glued to the skull with a tissue adhesive, Ethicon Bucrylat (pins with screw-heads No. 1 and 3).

After installation of the single shear pins, the animals were filmed during feeding with an Arriflex 16-mm camera by a Siemens X-ray apparatus (Tridoros optimatic 800, with a Sirecon-2 image intensifier, 48–56 kV, pulse duration 1 ms) equipped with a 70-mm lens. The resolving power was grid-controlled, and an error of 0–2% was established. Recording was on Agfa Gevapan 30 negative film at 50 frames/s. The cinefilms were later analyzed frame by frame with an 'Old Delft' variable speed analytical projector. The positions of the pins were traced on single sheets, or the coordinates of the markers were established with a digitizer for further analysis.

Application to the study of mastication

Implantation of TMS® pins as markers for cineradiography in small objects permits an exact measurement procedure. The use of these pins in the mandibles of the small shrews allowed a precise analysis of their movements from cineradiographic films. The screw-head and the end of each pin are independent markers. We thus used six points in a jaw to reconstruct a model of the movement pattern. A chewing cycle includes both opening and closing movements.

The positions of the two lower halves can be traced frame by frame on separate sheets; these sheets are subsequently superimposed into a single plot. A line connecting the screw-head of the posterior pin on the skull (fig. 3, No. 1) and that of the anterior marker (point No. 3) was used as a reference for the superimposition of the respective sheets. The connection of the markers of a pin then results in a model of a movement profile of the lower jaw (fig. 3, left jaw) during one masticatory orbit, derived from 12 frames in lateral view. For the sake of clarity, only the occlusal and the maximal jaw opening position are indicated as a 'three-dimensional solid body'. The solid lines represent the opening phase, the figure with the points Nos. 9', 11', and 13', shows the maximum opening position, the dotted lines the closing phase of the cycle. Length and direction of the pins change in the successive frames of the masticatory orbit. Rotation and translation are visualized and thus indicate the movement in the third dimension. The irregular pattern of the successive pins during the chewing cycle suggests velocity and acceleration of the mandible. This reflects functional aspects of chewing, i.e., changes in the phase durations of the cycle. A procedure to represent chewing cycles derived from films in dorsoventral projection can establish the movement of both mandibles during ipsilateral and contralateral chewing. A problem arises with the simple tracing technique because the movements of the head cannot be subtracted.

The determination of the coordinates of the pin markers with a digitizer provides an improvement for further analysis. With regard to future publications on this topic we only allude to the principle met in such an analysis. The X and Y coordinates of implanted pin markers are established from each lateral and dorsoventral X-ray photograph (fig. 2., displaced at an angle of 90°) with a CALCOMP 2500 digitizer. A PC program then calculated the X/Y/Z coordinates using the X and the Y coordinates of each marker from those photographs. By displacing the two-dimensional X-ray photographs at an angle of 90°, skull movements can thus mathematically be corrected.

After that the markers on the two-dimensional frames of the cineradiographic films are digitized. Again, another computer program rotates the three-dimensional coordinates to the lateral or dorso-ventral projection of the film photograph and recalculates the X/Y/Z coordinates for the markers on the film pictures considering the magnification and the rotation. For this calculation, a tolerance of error of 5% was accepted. The three calculated coordinates of the markers on the film photographs are then graphically presented on the computer in dorsoventral, lateral and frontal views. The PC also displays the movements on the screen. Distances from the pin points, and distances from a line between two points and another

point, and angles between two lines are calculated. Changes of the respective distances or angles in a series showing the moving jaws during mastication thus reproduce the movement pattern.

Efficiency of pin implantation

The use of dental pins as markers in cineradiography allows an exact procedure in measurements and analysis in small animals. Their standardized size, and shape, and the stability of their position, make all marks comparable, which is an advantage over the amalgam or lead markers which were formerly used. Moreover, from one pin two marks are established. This is especially important for the fixation of markers in small objects. The best measurable point can thus be chosen. Simultaneous application of several pins per moving element allows determination of more marker points, thus decreasing the error of interpretation. Furthermore, the reproducibility of measurements is greatly enlarged because the form of the pins is stable.

The use of a hand driver with the pin and the linked plastic shank is very effective for surgical installation in small objects. Screws, as have been applied in *Tenrec*[6] or friction grip pins[11,12] are difficult to manipulate.

The dental gold plated pins do not produce adverse tissue reactions. For normal implantation into the compact bone adhesive, which often damages the cells, is not necessary. Therefore, pins can remain in the animal for life. Our observations in living animals show that the pins are encapsulated within a few days so the surrounding chewing muscles and vessels are soon protected against friction.

We expect that the implantation of self-shearing pins will also be useful in other movement studies using cineradiography for example studies of rib movements during respiration, or gill arch movements of fishes.

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Blood pressure and impairment of endothelium-dependent relaxation in spontaneously hypertensive rats

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Summary. Correlation between hypertension and impairment of endothelium-dependent relaxation was demonstrated using aortae from certain strains of rats with various levels of spontaneous hypertension. It was also observed that the impairment of endothelium-dependent relaxation is the secondary change due to hypertension, and the level and duration of hypertension is the determinant factor of the impairment.
Key words. Spontaneously hypertensive rats; strains: ages; aorta; endothelium-dependent relaxation.

Endothelium-dependent relaxation in animal models of genetic hypertension has been reported to be impaired as compared with that of control normotensive animals[1–11]. This may indicate the involvement of a genetically-induced impairment of endothelium in the initiation of hypertension. However, the decreased endothelium-dependent relaxation can also be observed in thoracic aortae that have been made hypertensive by coarctation[11]. In addition, the decreased endothelium-dependent relaxation of blood vessels of hypertensive rats can be normalized by antihypertensive treatment[8,11]. These two reports[8,11] indicate that impairment is secondary to elevated blood pressure. Thus, the contribution of decreased endothelium-dependent relaxing activity in the initiation of hypertension is still controversial. In the present study, the age-related impairment of endothelium-dependent relaxation was studied using different strains of spontaneously hypertensive rats with various levels of blood pressure. All of the strains used in the present study are genetically established from the same origin; Wistar Kyoto rats (WKY)[12]. We report here that the impairment of endothelium-dependent re-