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Assessment of damage from implantation of microdialysis probes in the rat hippocampus with silver degeneration staining

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We used a sensitive silver degeneration staining method to study the effects of insertion of microdialysis probes in rat dorsal hippocampus and neocortex. Nine animals were sacrificed 24 h, 3 days or 7 days after implantation of dialysis tubing. Although mild neuronal cell death and small petechial hemorrhages were seen in close proximity to the implantation site, the striking finding was the presence of degenerating axons both adjacent to the implantation site and in remote sites such as the corpus callosum and contralateral hippocampus. The observed changes could alter brain function near or remote from the implantation site and should be considered in analysis of dialysis experiments.

Recent technological advances in the design and implementation of miniaturized dialysis probes have allowed in vivo measurements of a wide variety of substances in the brains of living animals. Since some brain damage invariably occurs when an object is inserted into cerebral tissue, knowledge of the pathologic effects of implanting such probes is important for interpreting the results of dialysis experiments.

Localized tissue reactions to the implantation of 'foreign bodies' have been described in the past. They include neuronal damage, microglial and astrocytic reactions [2], localized hematomas [12], alterations in glucose metabolism and the blood brain barrier [1, 10] and local biochemical disturbances. These changes may be delayed after implantation. For example, glucose metabolism decreases 24 h after implantation of dialysis probes [1], and glial reactions usually begin 2 or 3 days after implantation [2]. The extent of implantation damage is usually considered small and

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dependent on several factors including the size and shape of the instrument, its chemical composition, and the duration of its implantation.

An additional type of implantation damage which has not previously been considered is the interruption of fibers of passage. Severed axons may belong to neurons remote from the insertion site and may project to either nearby or distant brain regions. We therefore used a silver degeneration staining method to study the extent of axonal damage after implantation of microdialysis probes into the rat hippocampal formation. This staining technique preferentially deposits colloidal silver in injured neurons and their processes [5-7] and has been used previously to define projections of specific neuronal groups [5] and to study neuronal damage due to ischemia [4] and neurotoxins [8].

For these experiments the dialysis tube (Fig. 1) was constructed from a 9 mm length of hollow dialysis fiber (Allegro H.F. disposable dialyzer, Organon, Durham NC). A stainless steel wire was inserted into the dialysis fiber which was then connected at both ends to fused silica capillary tubing (0.17 mm outside diameter) with a small amount of Super Glue. The dialysis fiber was then bent into a loop over the tip of a hypodermic needle. The open ends of the capillary tubing were pushed into a 1.5 cm length of 20 gauge stainless steel tubing, pulled until the exposed tip of the dialysis loop was 2 mm from the edge of the steel tube, glued to the steel tube and connected with glue to PE-10 polyethylene tubing.

Next a 1.7 cm length of 16 gauge stainless steel was slid over the 20 gauge steel tube and the free end of the PE-tubing and glued to the 20 gauge steel tube. This outer steel tube prevented the fused silica capillaries from breaking at their junction

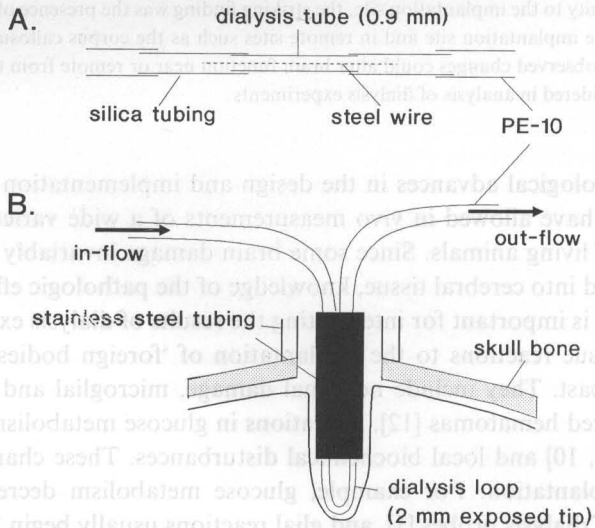


Fig. 1. Diagram of the dialysis probe used in this study. A: a 1.5 mm length of thin steel wire is threaded through a 0.9 mm length of dialysis fiber. The dialysis fiber is then connected at both ends to silica tubing attached to PE-10 polyethylene catheters. This drawing demonstrates the extended fiber and tubing before insertion. B: the tubing is bent to form a loop with a 1 mm diameter and a 2 mm length of exposed dialysis surface. In this drawing the complete probe is shown as inserted into the skull.

with the PE-tubing. The PE-10 tubing was then threaded through a 22 cm length of a PE-240 tube to complete the construction of the cannula. The outer diameter of the dialysis implant was one mm in diameter, which is approximately twice the size of commercially available dialysis probes [11].

Nine male Sprague–Dawley rats (250–330 g) were anesthetized with ketamine (130 mg/kg) and acepromazine (1.3 mg/kg) and placed in a stereotaxic apparatus. Dialysis probes were placed in the hippocampal formation, using a stereotaxic apparatus, with the incisor bar at 3.3 mm below horizontal zero and the following bregmatic coordinates: posterior 4.2 mm, lateral 3.2 mm, ventral (from the dural surface) 3.8 mm. The probes were fixed to the skull with small stainless steel screws and dental acrylic and the animals were allowed to recover from surgery for 24 h, 3 days and 7 days.

At 24 h ($n=3$), 3 days ($n=2$), or 7 days ($n=4$) after surgery, the animals were anesthetized with sodium pentobarbital and perfused through the heart with 10% formalin in 0.1 M phosphate-buffered saline, pH 7.4. The dialysis probes were carefully removed after the brains were fixed *in situ* and the brains were then removed from the skull and conserved in fixative for an additional 7–21 days. The fixed brains were cryoprotected by immersion in 30% sucrose in 10% buffered formalin for 48 h and coronal sections (50 μm) were cut through the forebrain with a freezing microtome.

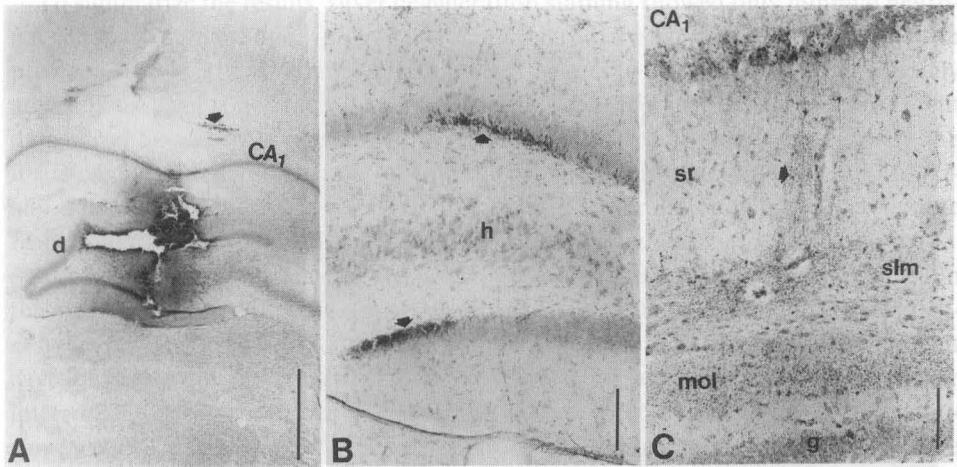


Fig. 2. Photomicrographs of sections taken adjacent to insertion sites of microdialysis probes and stained with a silver degeneration technique. A: low power photomicrograph showing microdialysis site in an animal sacrificed 7 days after probe insertion. The arrow points to degenerating neurons in overlying cortex adjacent to the insertion tract. The bar indicates 1 mm. B: arrows point to degenerating granule cells in the dentate gyrus adjacent to a microdialysis probe implanted for 3 days. The bar indicates 100 μm . C: photomicrograph through CA1 and adjacent dentate gyrus from an animal sacrificed 24 h after microprobe insertion. The arrowhead points to a small column of degenerating dendrites in stratum radiatum adjacent to the implantation site. Degenerating terminals can easily be seen in the stratum lacunosum moleculare of CA1 and the outer two-thirds of the molecular layer of dentate gyrus. The bar indicates 100 μm . Abbreviations: CA1, CA1 region of the hippocampal gyrus; d, dentate gyrus; mol, molecular layer; slm, stratum lacunosum-moleculare; sr, stratum radiatum.

One section at each level corresponding to plates 27, 29, 32, and 34 in the atlas of Paxinos and Watson [9] was stained by a silver impregnation method to label degenerating neurons and their processes [5, 6]. These levels spanned a distance of approximately 2 mm rostral to caudal, with the dialysis probe in the middle. At each level, adjacent sections were stained with cresyl violet, a Nissl stain used to define neuronal types and cytoarchitectonic regions.

The neuronal damage adjacent to the probe insertion site is shown in Fig. 2, while more distant damage is illustrated in Fig. 3. The tracts of the dialysis implants were clearly visible in all brains studied and extended from the cortical surface to the dorsal hippocampal formation (Fig. 2A). Although 4 of the animals had organizing small petechial hemorrhages adjacent to the implant, little neuronal loss or gliosis was apparent in the Nissl stain. Nonetheless, argyrophilic neurons were easily found up to 100 μm from the tract in both the neocortex and the hippocampal formation (Fig. 2B). In addition, fine silver deposits, probably representing both degenerating neurites and nerve terminals, were present up to 200 μm from the probe tract (Fig. 2C). Degenerating axons were seen in the cortical white matter and were present in the corpus callosum at the level of the implant (Fig. 3C).

The most dramatic changes seen in the silver stain were in the hippocampal formation where well-defined pathways were clearly interrupted. Axonal damage was evi-

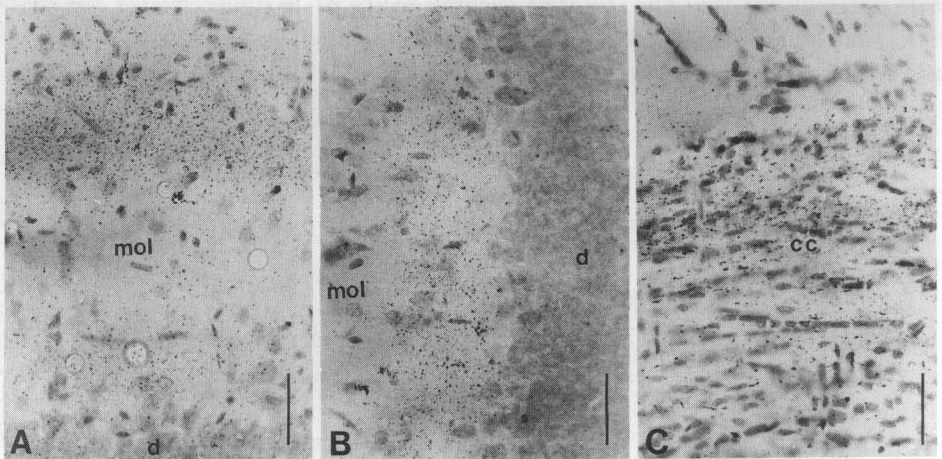


Fig. 3. Photomicrographs of silver-stained sections distant from the microdialysis probes. The bars indicate 50 μm in all 3 micrographs. A: photomicrograph from an ipsilateral section approximately 1 mm distant from the insertion site showing silver granules in the stratum lacunosum-moleculare of CA1. By contrast only scattered silver granules are present in the molecular layer, most prominently in the inner third. This section is taken from an animal sacrificed 3 days after probe insertion. B: nerve terminal degeneration in the hippocampus contralateral to the insertion of a dialysis probe. The degenerating terminals are predominantly in the inner third of the molecular layer of the dentate gyrus. The section is taken from an animal sacrificed 7 days after implantation. The photograph is rotated. C: section through the corpus callosum of an animal sacrificed 7 days after microdialysis probe implantation. Degenerating fibers are seen remote from the implantation site. Abbreviations: g, granule cells; mol, molecular layer; slm, stratum lacunosum moleculare; cc, corpus callosum.

dent on the side of the dialysis probe in all 3 of the animals examined 24 h after implantation and became increasingly prominent with longer survival times. In almost all animals, there was both anterograde and retrograde degeneration in the Schaffer collateral fibers in stratum radiatum and stratum oriens at the level where the implant had transected the pathway. Similar degeneration was present in the mossy fibers, with argyrophilic axons visible in both the hilus of the fascia dentata and in the stratum lucidum of area CA3. Degenerating axons were present in the projection zones of the entorhinal cortex, including the outer two-thirds of the molecular layer in the fascia dentata and the stratum lacunosum-moleculare in areas CA1–CA2.

Implantation of the dialysis probe also interrupted commissural fibers. In one of the 3 animals sacrificed at 24 h, the silver method showed faint staining in the stratum radiatum and stratum oriens of the contralateral CA1 and in the inner blade of the molecular layer of the contralateral dentate gyrus. This contralateral damage was apparent in both animals examined at 3 days and was particularly prominent in the 4 cases studied 7 days after implantation (Fig. 3B). No contralateral argyrophilia was present outside the termination zones of the commissural pathways.

The outer diameter of the dialysis probe was 1 mm in our model. This is approximately double the size of other commonly used similar probes [11]. Thus it is possible that small probes may interrupt fewer fibers. However, push-pull cannulae are of the same diameter as the probes used in our study [12].

To summarize the results, silver degeneration staining showed only minimal neuronal cell body damage after implantation of a microdialysis probe. In agreement with previous studies, the cell damage was limited to one to two cell layers immediately adjacent to the probe tract. More strikingly, we have shown mild but consistent damage to fibers of passage transected by the probe. In our material, degenerating fibers could be seen at sites remote from the probe both ipsilaterally and contralaterally. The abnormalities of glucose metabolism and cerebral blood flow in sites remote from dialysis probes [3] could be secondary to this axonal loss.

The biological significance of this interruption of axonal pathways is not clear. On the one hand, these pathways were only partially transected at one point. On the other hand, degenerating fibers were present in sections rostral and caudal to the implant cavity and also in the contralateral hippocampal formation and neocortex. Some interrupted fibers were associational and would be expected to influence firing of neurons near the dialysis probe. Fibers degenerating in remote areas could also influence neuronal activity near the probe through various feedback systems. Such possibilities must be considered when interpreting the results of dialysis experiments.

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