

TECHNIQUE FOR INSERTION OF A PUSH- PULL CANNULA WITHIN THE ANTERIOR PITUITARY GLAND IN THE PIG

Richard Barb, Ph.D.

Steven Leshin, Ph.D.

Robert R. Kraeling, Ph.D.

Animal Physiology Research Unit

Richard B. Russell Agricultural Research Center

USDA-ARS

Athens, GA 30613

George B. Rampacek, Ph.D.

Animal and Dairy Science Department

University of Georgia

Athens, GA 30602

Dr. Barb received his Ph.D. in animal science from the University of Georgia in 1985 and is currently a research physiologist in the Animal Physiology Research Unit. Dr. Kraeling received his Ph.D. in animal science from Iowa State University in 1970. He currently is research leader for the Animal Physiology Unit. Dr. Leshin received his Ph.D. in animal and dairy science from Purdue University in 1983. He is currently a physiologist in the Animal Physiology Research Unit. Dr. Rampacek is currently a professor in the Animal and Dairy Science Department at the University of Georgia. He received his Ph.D. in animal science from North Carolina State University in 1974. Dr. Barb can be reached at 706-546-3226

INTRODUCTION

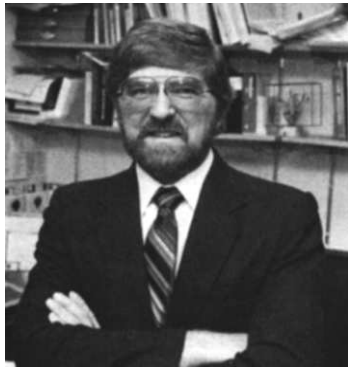
Identifying specific components of the central nervous system which modulate pituitary hormone secretion has been the subject of intensive investigation in recent years. Understanding these mechanisms in domestic farm animals is essential for enhancing reproduction, lactation and growth.

While small laboratory animals may provide an easier, workable "model system" for some research problems, there are sufficient differences among species so that results from model systems that should be confirmed or verified in the actual animal of interest. One obstacle in neuroendocrine studies of reproduction and growth in pigs is lack of access to the hypothalamus and pituitary gland due to species differences in skull anatomy. This report describes a reliable method to insert a push-pull cannula (PPC) into the anterior pituitary gland of the pig using stereotaxic techniques.

MATERIAL AND METHODS

Animals are anesthetized with a 10% thiopental sodium and placed in ventral recumbency. After endotracheal intubation, surgical anesthesia is maintained on a closed circuit system of halo-thane and oxygen. The forehead is shaved and prepared for surgery by aseptic techniques. A push-pull cannula guide (CG) is stereotaxically positioned, with the aid of radiograms, within the pituitary gland (Figure 1) using procedures modified from Levine et al. (1) and Estienne et al. (2). The head is placed in a modified Kopf stereotaxic holder as described by Barb et al. (3) such that the alveolar process of the maxilla is aligned parallel to the stereotaxic A-P bars. This maintains the jugum-sphenoidal, above which lies the ventral forebrain, at a 10-20° slope from the horizontal plane (Figure 1). For insertion of cannulae into the pituitary gland, an approach perpendicular to the plane of the dorsal surface of the pituitary allows for a larger "target region" than an angled approach as described for insertion of cannulae into the lateral ventricle (3). More importantly, a perpendicular approach avoids penetration and damage of parts of the hypothalamus and median eminence which produce and convey neurohormones to the pituitary gland. From a lateral radiogram, the position of the CG is determined such that its tip would lie within the anterior pituitary gland approximately 3-6 mm posterior and 3-5 mm ventral to the tuberculum-sellae. A hole, perpendicular to the horizontal plane, is drilled through the sagittal suture. The

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Editor's Column

It is a fairly warm, overcast day in Kansas City in mid December as I write this column. I hope for some snow for Christmas, but also hope it isn't too nasty. Isn't it amazing how we wish for

things we can't influence? Maybe we should take more time to think about the things we can influence and how.

One of the things we can influence is the publicity of science. There is a lot of good publicity coming out now about the advances in our understanding of how the brain and nervous system work, and how to treat various disease states. Almost every day, we see news items telling about some recent discovery in brain function, or in cancer treatment. However, it seems that we are not yet getting across the message to the public about the necessity for basic science in all these discoveries and insights. One of the problems is the continuing barrage of public disinformation and distortions put out by some of the animal rights groups on the importance of basic research in the process of gaining knowledge. Another is the public impression from some widely publicized cases, that waste and improper practices are widespread in science.

It is imperative that scientists working in their own labs realize that their interests are at stake in the continuing discussions on animal rights, funding and control of science. We all must be willing to stand up for what we believe in the areas which we can influence, and not take an attitude of "let the other guy do it."

As 1994 begins, it brings with it many opportunities and many possibilities. Hopefully, we will all renew our commitment to seizing opportunities to help others understand the vital role of basic research in the unfolding development of our place in the universe.

HAPPY NEW YEAR

Michael M. Patterson, Ph.D.

Science Editor
College of Osteopathic Medicine
The University of Health Sciences
2105 Independence Blvd.
Kansas City, MO 64124-2395
816-283-2308
FAX 816-283-2303

CG is lowered through the sagittal sinus into either the third ventricle for ventriculography (about 8 mm above the final position) or to a position 2-3 mm above the pituitary. Lateral ven-triculograms are obtained following withdrawal of .3 ml cerebrospinal fluid from the third ventricle and replacement with .3 ml radioopaque dye (Wintrop-Breon Lab., New York). For placement without the aid of ventriculograms, dorsal radiograms are helpful to determine lateral position. Figure 1 illustrates final placement of the CG in a representative animal. Bone screws are placed into adjacently drilled holes and the CG is secured to these screws with dental acrylic. Postoperative prophylactic treatment consists of penicillin G and oxytetracycline.

Push-Pull Cannula and Cannula Guide. The CG consists of a 17-gauge stainless steel tube with an occluding inner 20-gauge stainless steel solid rod stylet that protrudes 2 mm from the tip of the outer tube. The PPC consists of two concentric stainless steel tubes; a 20-gauge outer tube and an inner 26-gauge tube which protrudes 1.5 mm beyond the tip of the outer tube. Lengths of cannulae are determined from lateral radiograms of anesthetized pigs about one week before surgery.

General Procedures. Following surgical recovery, animals are habituated to Panepinto slings (4) modified to fit large pigs (3). Slings limit movement, but still allow animals to stand or lie, drink and feed. The slings allow pigs to be raised off the floor for brief periods to minimize head movements. The day prior to blood sampling, a catheter is placed by percutaneous puncture into the jugular vein. On the day of the experiment, the CG stylet is removed and replaced with PPC connected by PE20 polyethylene tubing to two identically balanced peristaltic pumps. Sterile filtered Krebs-Ringer phosphate buffer containing .2 mM bacitracin (Sigma Chemical Co., St. Louis, MO) is infused through the inner cannula and withdrawn through the outer cannula at 20 ul/min (Figure 2). Fractions collected over 10 min intervals are quickly frozen. Blood samples are taken at the midpoint of perfusate samples.

DISCUSSION

This procedure has been successfully used for assessing the temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in the pig (5). Profiles of GnRH and LH revealed that both hormones are secreted in pulsatile patterns with

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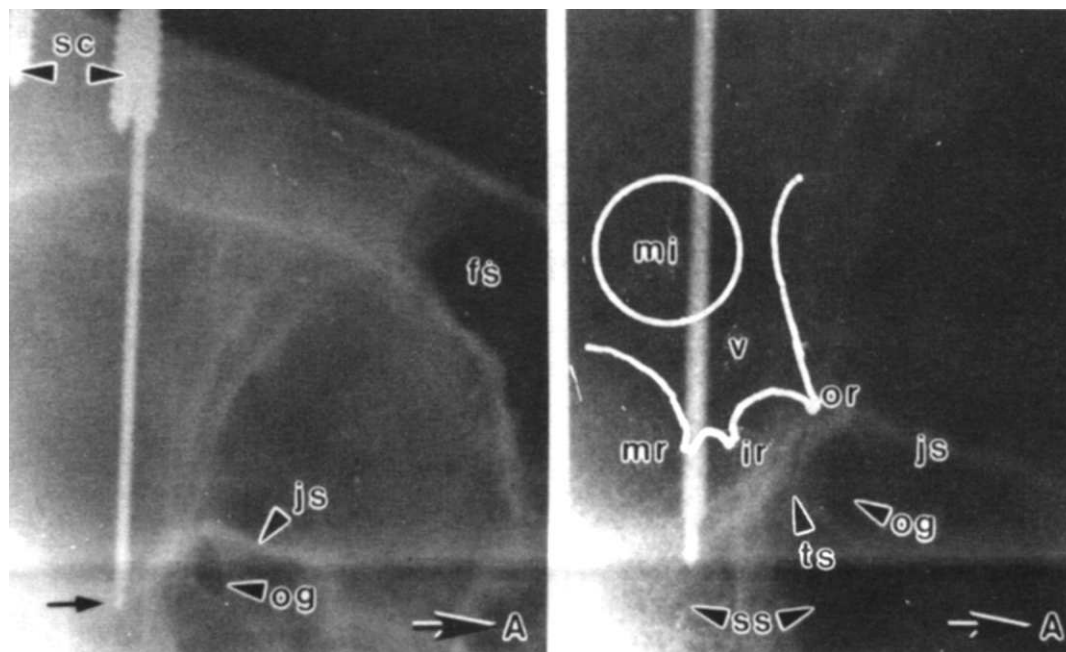


Figure 1. Left lateral radiogram of a representative pig head with tip (small arrow) of the push-pull can-nula guide located within the pituitary gland before cementing to screws (sc) inserted within the frontal bone. Right, enlargement of region containing cannula tip and ventral skull structures. Tracing of third ventricle (in white) is overlaid on this radiogram. Abbreviations: A, anterior direction; fs, frontal sinus; ir, infundibular recess of third ventricle; js, jugum sphenoidale; mi, massa intermedia; mr, mam-millary recess of third ventricle; og, optic groove; or, optic recess of third ventricle; sc, bone screws; ss, sphenoidal sinus; ts, tuberculum sellae; v, third cerebral ventricle. (From Leshin, et al, (5), reproduced with permission of Butterworth-Heinemann)

peaks of GnRH often, but not always, occurring coincident with or just preceding LH peaks (Figure 3).

The advantage of locating the PPC in the pituitary is the potential for detection of the same hypothalamic signal which is perceived by pituitary cells. Large animals might be advantageous compared to small laboratory animals in some cases. In large animals, the cannula occupies and thus damages, a relatively small part of the pituitary gland, leaving the vast majority of the gland functioning normally and undisturbed.

A major disadvantage of the push-pull technique for characterization of hypothalamic GnRH is the variable and unpredictable amount of dilution of the neurohormone sampled, requiring a highly sensitive assay. With the PPC technique, there is exchange and dilution of extracellular concentrations of GnRH by the perfusing medium and thus, GnRH concentrations detected represent only a small fraction of hypophysial portal blood concentrations. In conscious ewes, either ovariectomized or during the LH surge, profiles of GnRH secretion from PPC perfusion of the median eminence (6,7) were similar to those profiles obtained from hypophysial portal blood collection (8,9). Therefore, our sampling of pituitary extracellular fluid of the pig is probably a

diluted but accurate reflection of the hypothalamic signal necessary for LH secretion. The high correlation of pulses of GnRH in PPC per-fusates with pulses of LH in peripheral blood obtained from conscious unstressed pigs attests to the usefulness of this technique.

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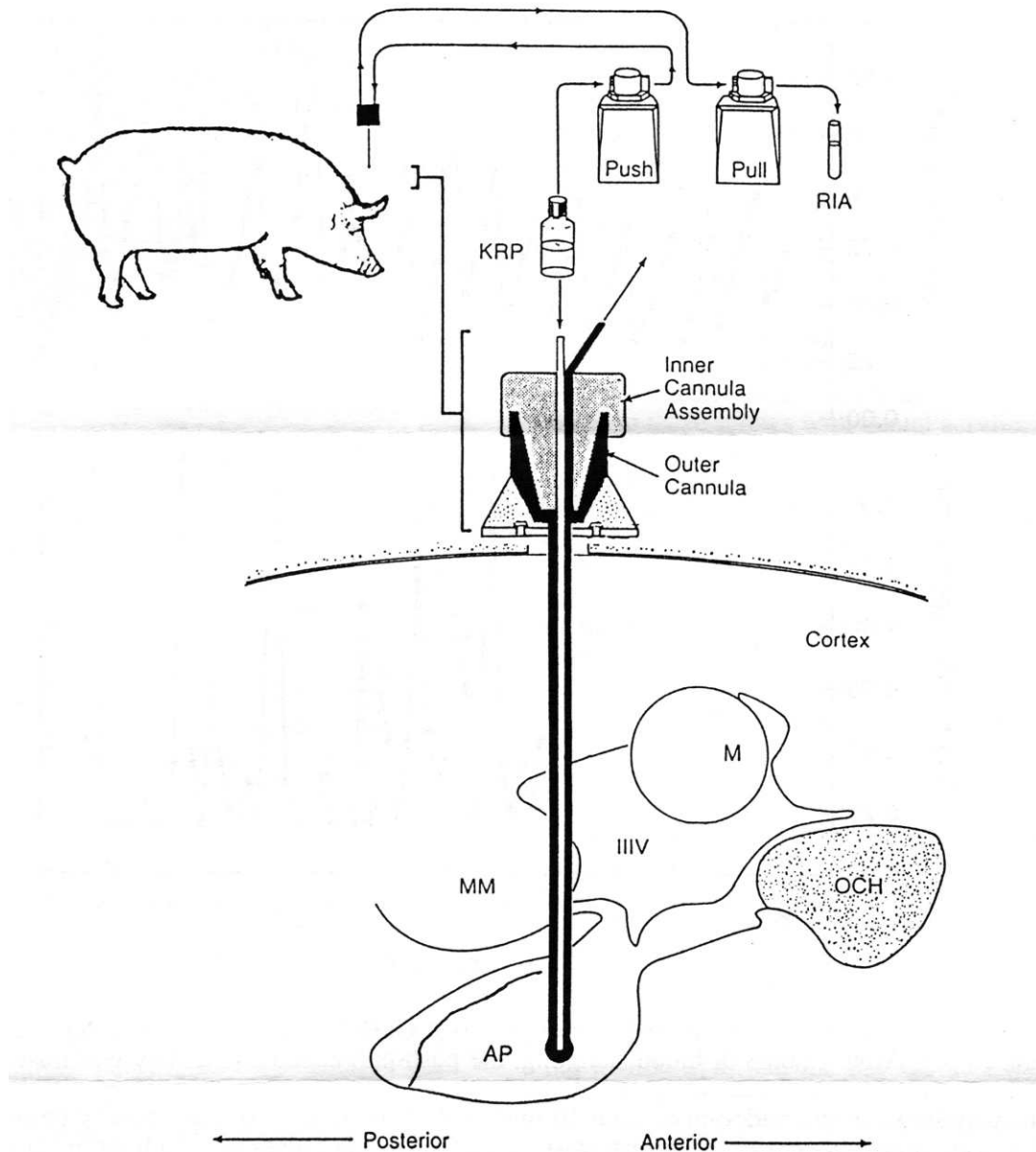


Figure 2. Schematic illustration of the push-pull perfusion technique. A push-pull cannula assembly replaces the guide cannula stylet, with the tip positioned within the anterior pituitary (AP) gland. Perfusion media (Krebs-Ringer phosphate buffer containing .2mM bacitracin; KRP) is pumped ("pushed") through narrow gauge tubing and the inner cannula at 20 ul/min. At the cannula tip this media mixes with extracellular tissue fluid. A second pump "pulls" at precisely the same rate, the mixed extracellular fluid/ perfusion media through the outer cannula into collecting tubes changes every 10 min. These fractions of perfusate are assayed for neuropeptides such as GnRH by radioimmunoassay (RIA). Abbreviations: M, massa intermedia; MM, mammillary body; OCH, optic chiasm; III V, third cerebral ventricle.

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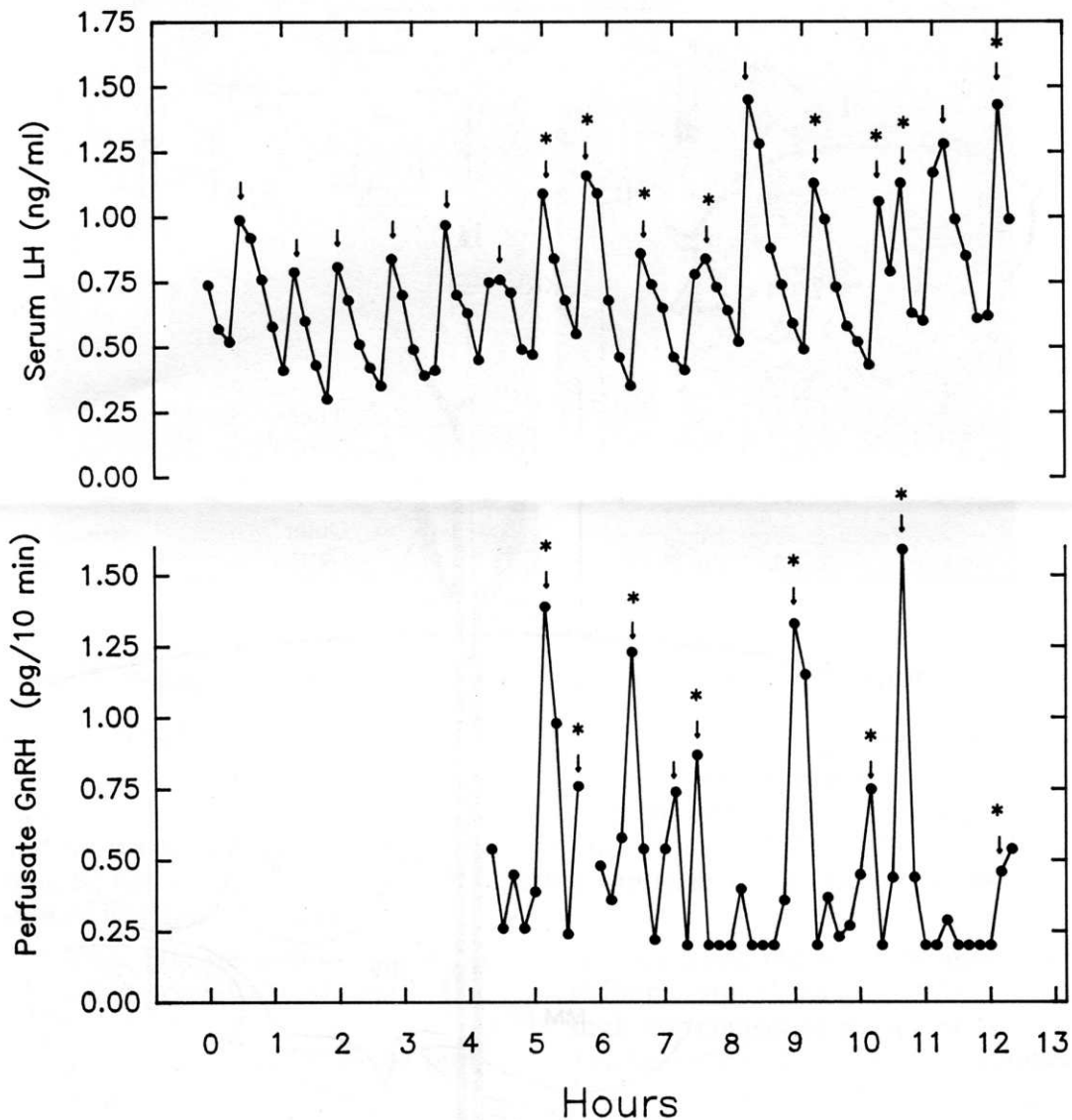


Figure 3. A secretory profile of serum LH and perfusate GnRH concentrations in a representative ovariectomized pig. After 4 hours of blood sampling, the push-pull cannula assembly was installed through the guide cannula and perfusate samples collected continuously by peristaltic pump with jugular blood samples withdrawn at the midpoint of each 10 min. collection period. Arrows identify peaks of LH and GnRH pulses. Stars above arrows indicate associated GnRH-LH pulses in which a GnRH pulse occurs coincident with or one sample prior to a LH pulse. (From Leshin et al, (5), reproduced with permission of Butterworth-Heinemann).

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