Pharmacology Biochemistry & Behavior, Vol. 6, pp. 471–474. Copyright © 1977 by ANKHO International Inc.
All rights of reproduction in any form reserved. Printed in the U.S.A.

BRIEF COMMUNICATION

A Modified Push-Pull System for the Localised Perfusion of Brain Tissue

PETER REDGRAVE[1]

Department of Psychology, The University, Hull, England

(Received 11 November 1976)

REDGRAVE, P. *A modified push-pull system for the localised perfusion of brain tissue.* PHARMAC. BIOCHEM. BEHAV. 6(4) 471–474, 1977. A method for monitoring the outflow of a push-pull perfusion system is presented. This modification possesses the following important advantages: (1) Inadvertent induction of expansion lesions is avoided, (2) Flow rates can be reduced from the usual 50–200 μl/min to 0.6–1.7 μl/min without decreasing the probability of detecting occlusions, (3) Incidence of occlusions is reduced, (4) Up to 30–40 perfusions can be performed at each of several sites in the brain of a single animal over a period of several months. The modified system has been used successfully to administer neurohumoral agents to the brain of rats and to determine the release of endogenous substances into the perfusate.

Push-pull perfusion Outflow monitor

THE INITIAL concept of the push-pull perfusion which was introduced by Fox and Hilton [3] was later adapted for the perfusion of discrete regions of the brain by Gaddum [4]. Theoretically, the perfusion fluid is pushed through one cannula and is recovered by negative pressure applied to a second cannula. This is most commonly achieved [6] by connecting the cannulae, by means of polyethylene tubing, to two syringes mounted in a reciprocally acting infusion-withdrawal pump. The relative positions of the push-pull cannulae in brain tissue can be either side-by-side as described by Delgado [2] or one inside the other, as in the original concentric arrangement proposed by Gaddum [4]. Both designs permit a discrete area of brain at the tip of the push-pull cannulae to be continuously bathed by the perfusion fluid. This technique can be used in two ways. Firstly, to administer neurohumoral or pharmacological agents directly to localised regions of the brain, and secondly, to determine the ongoing release of endogenous substances into the perfusate.

In practice, however, a small portion of brain tissue is sometimes sucked into the open lumen of the withdrawal or pull-cannula thereby effecting a complete or partial blockage of the cannula responsible for the removal of perfusate from the brain. When all air is excluded from a closed push-pull system the first warning of a blockage is either the appearance of vacuum bubbles in the pull-line, or the drawing of air into the back of the pull-syringe. The latter can be eliminated by applying a small amount of silicone grease to the plunger of the pull-syringe.

Initially, experiments were performed in order to determine the time taken for vacuum bubbles to appear following an artificial blockage of the pull-line in a closed push-pull system. The blockage was effected by clamping the pull-line with a pair of forceps. Table 1 presents the mean times taken to detect the first signs of such a blockage under conditions of varying perfusion flow-rate. Also included in Table 1 are the mean volumes of fluid which would be unavoidably pumped into the brain by the push-syringe during the time taken to detect an obstruction in the pull-line.

The unrecovered loss of fluid into the brain tissue of a preparation following a blockage of the pull-cannula places serious constraints on the subsequent interpretation of data, a point raised previously by Chase and Kopin [1] and by Izquierdo and Izquierdo [5] who question the value of results obtained from the perfusion of traumatized tissue.

The conceptual advantages and technical information concerning the push-pull perfusion technique have been described [6] although a method for preventing the loss of relatively large quantities of perfusion fluid following a blockage of the pull-cannula has not been reported. The purpose of the present communication is, therefore, to present a simple modification which provides instantaneous indication of occlusions occurring in the pull-side of the push-pull system.

The materials and techniques for constructing the push-pull system have been described in detail by Myers [6]. Briefly, the outer or pull-cannula was fashioned from 23-gauge stainless steel, thin-walled tubing while the inner or push-cannula was constructed from 29-gauge needle

[1] Reprint requests to: P. Redgrave, Department of Psychology, University of Sheffield, Sheffield, S10 2TN, England.

TABLE 1

TIME TAKEN FOR VACUUM BUBBLES TO APPEAR IN A CLOSED PULL-LINE FOLLOWING AN ARTIFICIAL BLOCKAGE AND THE VOLUME OF FLUID WHICH IS UNAVOIDABLY PUMPED INTO THE BRAIN DURING DETECTION TIME

Perfusion Flow Rate (μl/min)	Mean time taken to detect blockage in pull-line (min ± SE)*	Mean amount of fluid pumped into brain during detection time (μl ± SE)
7.9	3.43 ± 0.42	27.1 ± 3.3
19.7	1.83 ± 0.19	36.1 ± 3.8
39.3	1.32 ± 0.19	51.7 ± 7.7
79.0	0.80 ± 0.04	63.2 ± 2.8
197.0	0.38 ± 0.02	75.5 ± 3.7

*N = 5 observations at each flow rate.

tubing. Both cannulae were connected, by means of PE10 polythylene tubing, to 1 ml glass syringes mounted in a reciprocally acting infusion-withdrawal pump. Figure 1 illustrates the incorporation of a 3-way tap (Hamilton Valve 86503) and a calibrated monitor tube (PE10) into a normally closed push-pull system. The 3-way tap permits the pull-syringe (which is equal in volume to the push-syringe) to pull fluid either from the animal or from the monitor tube. Under normal circumstances, the total volume pushed through the system is pulled from the animal by setting the level of the fluid in the monitor-tube (ink marker) below the level of the animal. In fact, it is this hydrostatic pressure which creates the force required to extract perfusate from the brain. Now if the pull-cannula becomes blocked, the pull-syringe is no longer able to pull from the animal; instead, fluid is immediately pulled from the monitor tube. When this occurs the cannula assembly must be removed from the animal, and the material causing the blockage cleared by flushing before the perfusion is continued.

The advantages of this modification may be enumerated as follows:

1. Inadvertent induction of expansion lesions and experimental hydrocephalus is eliminated. Sections A and B in Fig. 2 were obtained from rats subjected to between one and five 15 min perfusion attempts with a closed push-pull system not possessing the present monitoring facility. In contrast, Sections C and D were derived from rats who had received between 10 and 25 push-pull perfusions of 20–30 min duration spaced over a period of several weeks; these perfusions were subjected to the present monitoring procedures. Clearly, the modified system enables the disruption of tissue in the brain to be restricted to the track along which the push-pull cannulae pass.

2. The amount of fluid unrecovered from the animal following an occlusion of the pull-cannula can be quantified by calibrating the monitor-tube, (usually, in 0.5 μl divisions). The unrecovered volume is indicated by the difference between the original and the final heights of the ink marker following an occlusion. A fall corresponding to 0.5–1.0 μl is considered sufficient grounds by the author to switch off the pump and determine the nature of the blockage.

3. The difference in height between the animal and the level of fluid in the monitor-tube provides a measure, (in

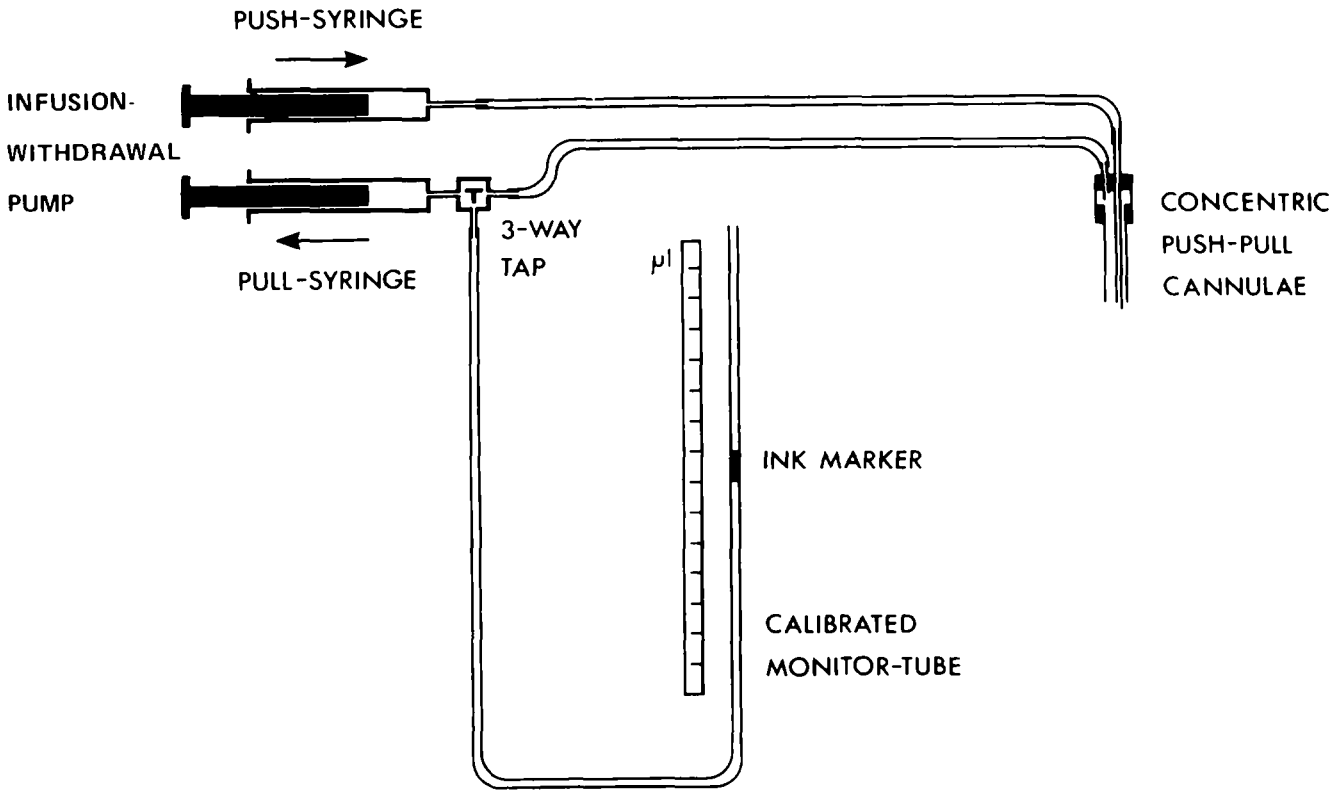

FIG. 1. Shows a schematic representation of the modified push-pull system. A three-way tap and a monitor tube have been incorporated into a normally closed push-pull system in order to facilitate the discovery of blockages in the pull-side of the push-pull system.

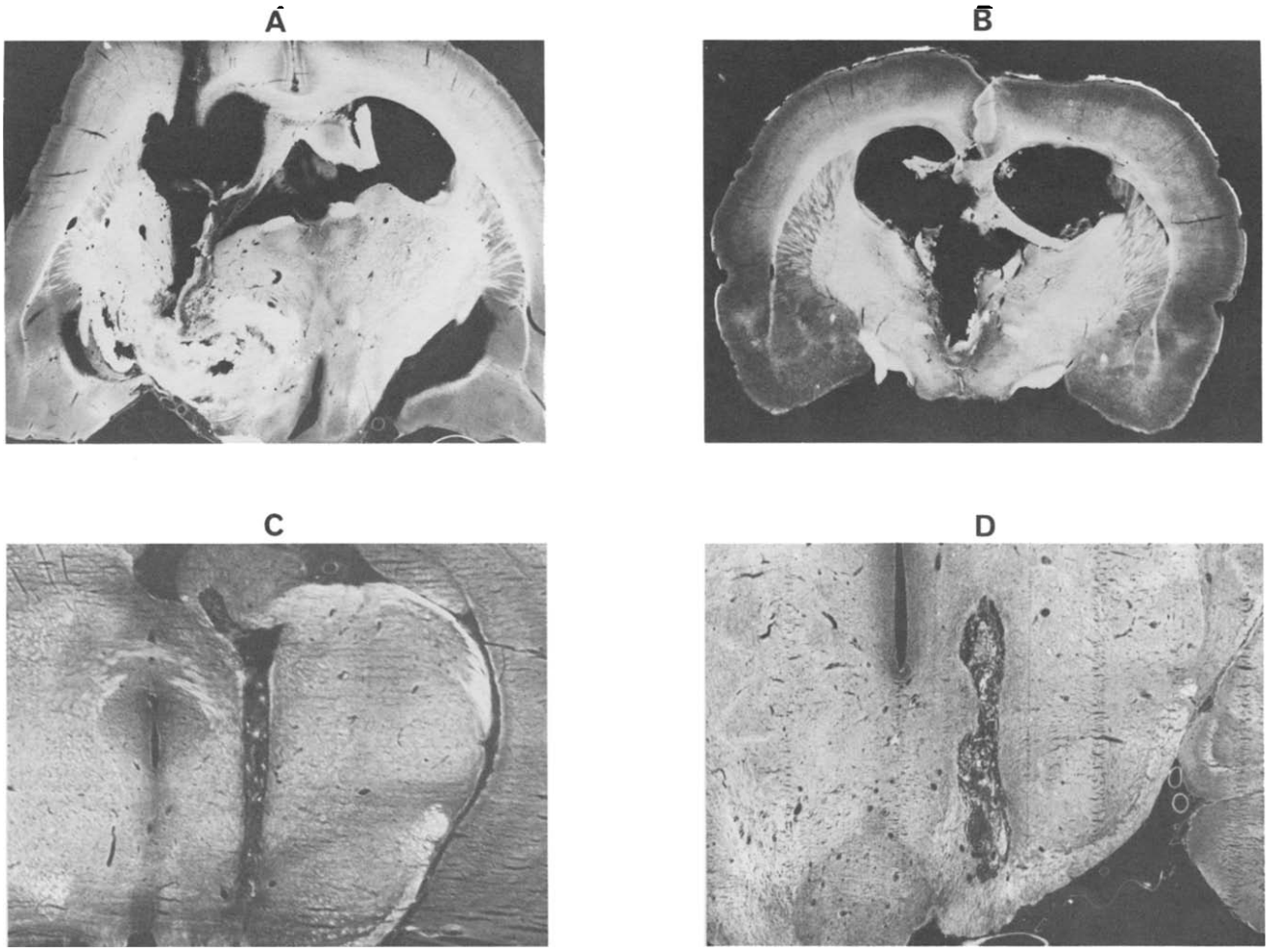

FIG. 2. Histological sections of rat brain. Sections A and B were obtained from brains subjected to push-pull perfusions in which a closed push-pull system was used. Sections C and D were obtained from brains subjected to similar perfusions except that the perfusion outflow was continuously monitored using the present modified system.

mm of water), of the pressure applied to the pull-side of the system. This is normally in the range of 10–300 mm of water.

4. The commonly used flow rates of 50–200 μl/min can now be reduced to 0.6–1.7 μl/min without decreasing the probability of detecting occlusions.

5. Flow rates of 0.6–1.7 μl/min are less likely to cause tissue erosion (i.e. mechanical damage) than flow rates of 50–200 μl/min.

6. The actual incidence of occlusions is reduced, firstly, by the diminished negative pressure applied to the pull-side of the system (i.e. 10 to 300 mm of water compared with an atmospheric pressure of approximately 10.33 metres of water when a blockage occurs in a closed pull-system) and secondly, by the greatly reduced flow rates.

7. The present monitoring facility permits the discovery of partial occlusions, that is, when less than the total volume pushed through the system is recovered from the brain. In such cases the fluid level in the monitoring-tube falls more slowly than when there is a complete blockage. In the present push-pull system partial occlusions often tend to clear themselves. However, in a closed system a partial occlusion would almost inevitably lead to a total occlusion as rapidly increasing negative pressure is applied to the pull-side of the system.

8. The viability of the preparation is greatly extended. Under ideal circumstances, which include an absence of haemorrhaging, few blockages, and successive perfusions separated by not more than 24–48 hr, as many as 30 to 40 perfusions can now be conducted at each of several sites in the brain of a single animal over a period of several months.

The present modified push-pull system is suitable for use with all common laboratory mammals, however, in rodents its use is particularly desirable because of the relative severity of lesions caused by unrecovered fluid loss in these animals. The present system has been used successfully in the establishment of dose-response relationships between centrally administered neurohumors and self-stimulation behaviour in rats [7]. The release of neurochemical agents into the perfusate has also been observed using the present modified system.

ACKNOWLEDGEMENTS

The author was supported in part by a David Ross fellowship of the Purdue Research Foundation and by the Science Research Council of Great Britain.

The author gratefully acknowledges the advice and support of Professor R. D. Myers in whose laboratory part of the present work was performed.

Some of the present information was included in a poster communication presented by the author at the First Neuroscience Meeting in Munich, W. Germany, (September, 1975).

REFERENCES

1. Chase, T. N. and I. J. Kopin. Stimulus-induced release of substances from olfactory bulb using the push-pull cannula. *Nature* **217:** 466 -467, 1968.
2. Delgado, J. M. R. Intracerebral perfusion in awake monkeys. *Archs int. Pharmacodyn. Thér.* **161:** 442 462, 1966.
3. Fox, R. H. and S. M. Hilton. Bradykinin formation in human skin as a factor in heat vasodilatation. *J. Physiol. Lond.* **142:** 219–232, 1958.
4. Gaddum, J. H. Push-pull cannulae. *J. Physiol. Lond.* **155:** 1 2P, 1958.
5. Izquierdo, I. and J. A. Izquierdo. Effects of drugs on deep brain centers. *A. Rev. Pharmac.* **11:** 189–208, 1971.
6. Myers, R. D., Methods for perfusing different structures of the brain. In: *Methods in Psychobiology*, Vol. 2, edited by R. D. Myers. New York: Academic Press, 1972, pp. 169 211.
7. Redgrave, P. and R. I. Horrel. Potentiation of central reward by localised perfusion of acetylcholine and 5-hydroxy-tryptamine. *Nature* **262:** 305 307, 1976.