PRO LABORATORIO

A simple apparatus for the rapid extraction and weighing of small animal brains

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Summary. The construction and use of an inexpensive apparatus for the rapid extraction and weighing of small animal brains is described.

Decapitation and delayed fixation lead to significant changes in the concentrations of several brain components[1]. Cyclic adenosine monophosphate (cAMP) is particularly sensitive in this regard: over a ten-fold increase occurring in some brain parts within 2 min of decapitation[2]. Even when a small animal is immersed in liquid nitrogen, up to 75 sec can be required for the core of the brain to reach 0 °C[3]. Focussed microwave irradiation is a fast convenient method for tissue fixation in situ, but the initial cost of the apparatus is rather high[2]. 'Freeze blowing' is quick and inexpensive, but is anatomically non-selective[4].

We have developed a simple and inexpensive method of quickly removing and denaturing whole rat brains or large brain areas. The device we use is fashioned from standard laboratory equipment and does not depend on the use of denaturing solvents (which can influence the protein-binding assay commonly used in the measurement of cAMP[5]). With the help of this method, the experimenter can produce a suspension of denatured brain of known wet-weight within less than 1 min. We have used the apparatus primarily in the determination of cAMP, but it is obviously adaptable for other purposes.

Procedure. One begins by pouring 25 ml of preheated buffer into the reservoir and 5 ml of the same buffer into the homogenizer (figure). The water in the bath on the hot plate is kept at 100 °C. Either dry ice/acetone or

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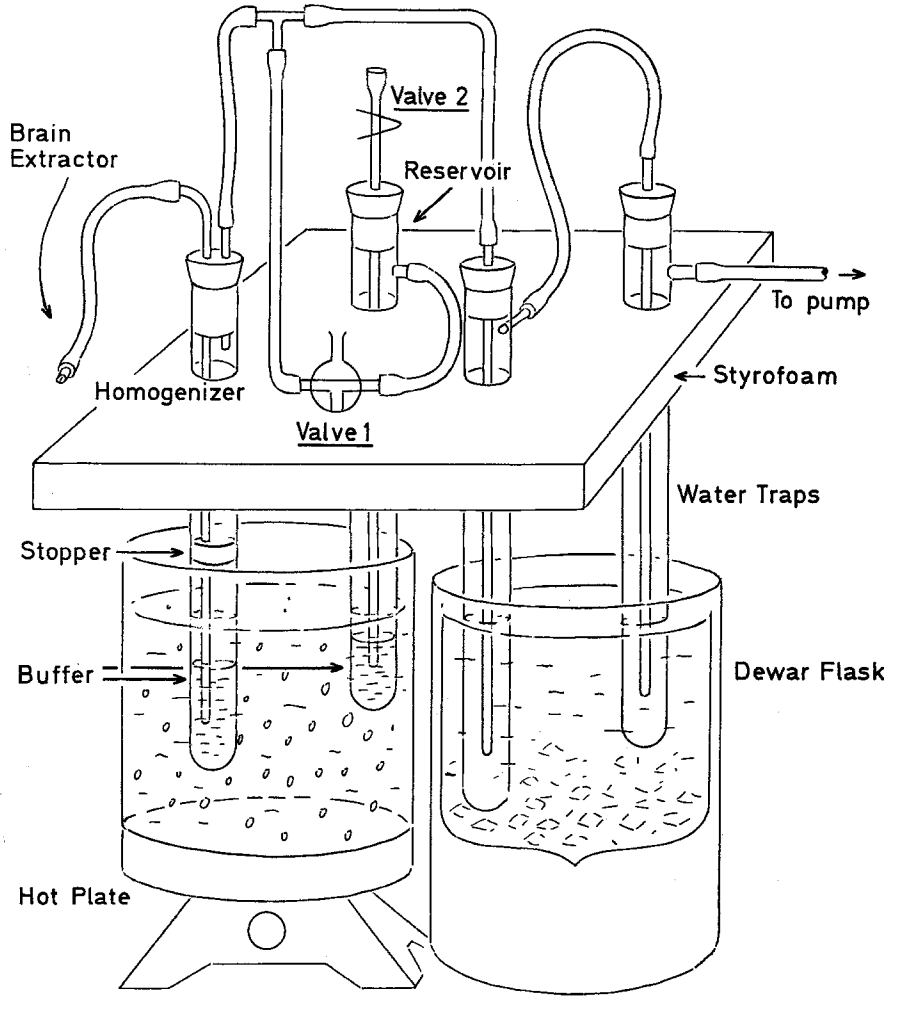

water/sodium chloride can be used as the coolant in the tandem water traps. (Dry ice/acetone is a slightly more efficient trap than water/sodium chloride, but a great deal more troublesome because of the problem of condensation and freezing of atmospheric moisture on the outside of the traps when they are removed from the freezing mixture to be weighed.) At the beginning of the procedure, valve 1 is set so as to connect the reservoir with the homogenizer (that is, as it appears in the figure). Valve 2 is clamped closed. The styrofoam-supported part of the apparatus is then removed from the baths, disconnected from the pump, dried off and weighed (accurate to 0.01 g) on a top-loading balance. The water traps are allowed to hang down on one side of the weighing pan, the homogenizer and reservoir hang down on the other side. The apparatus is then returned to its support (a ring stand is convenient) and the tubes replaced in the hot water bath and the contents of the Dewar flask respectively. The vacuum pump is reconnected (a water aspirator is sufficient) and turned on just before the rat is to be killed.

The rat is decapitated, and with a second stroke of the guillotine, the skull is split lengthwise and the brain or brain parts removed by aspiration with the brain extractor. Valve 2 is quickly turned so that the hot buffer in the reservoir is open to atmospheric pressure. The brain extractor is then inserted into the tubing above valve 2, thus sucking the buffer from the reservoir through the brain extractor tubing and washing the brain into the homogenizer. The aperture of the brain extractor can be chosen to give the proper combination of accuracy and speed. (The inverted stopper shown inside the homogenizer does not touch the inside walls of the tube; its purpose is to minimize upward splattering of the buffer. Its optimal position in the homogenizer must be determined by trial and error.)

With the brain suspended in the hot buffer, the apparatus is weighed again, the weight of the extracted brain being equal to the difference between the first and second weighings. The brain suspension is allowed to sit in the boiling water bath for a total of 5 min to insure complete enzyme denaturation. Either before, after, or during those 5 min, the homogenizer can be opened and its contents further homogenized with a teflon plunger. We have found wet weights obtained by this method are accurate to $\pm$ 2%. Adenyl cyclase (and presumably other enzymes as well) was over 90% denatured within 30 sec after decapitation.

PRO EXPERIMENTIS

Continuous registration of X, Y-coordinates and angular position in behavioural experiments

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Summary. An electronic camera and 2 simple additional circuits for the registration of the X, Y-coordinates or the angular position of animals in behavioural experiments are described.

In behavioural experiments, the problem of recording continuously the X, Y-coordinates or the angular position of an animal frequently occurs. For this purpose, electronic cameras are particularly suitable. To demonstrate their manifold possible applications, 2 simple circuits will be discussed which allow the registrations of the X, Y-coordinates of a running beetle and the rotation of a crab's eyestalks.

The output signal of the electronic camera. The camera objective Ob projects a white stripe S on a black background (figure 1) on to the semiconductive layer Sl of the image converter tube. An electron beam scanning the image on the layer Sl converts the light distribution line by line into a positive, analogue voltage called video signal. The temporal course of the video signal for 1 line corresponds to the distribution of the light intensity of the image on this line. In order to synthetize a TV-image by means of a monitor, the information about the beginning of the image and the lines has to be added to the video signal. The vertical synchronization pulse (negative, duration 110 µsec) signals the beginning of the image and causes the flyback of the vertical deflection. When the first horizontal synchronization pulse (negative, duration 4.5 µsec) reaches the monitor, the horizontal deflection starts and the light intensity distribution of the first line, corresponding to the video signal voltage, is displayed. The following horizontal synchronization pulses trigger the display of the subsequent lines. The video signal is blanked from about 3 µsec before to 3 µsec after the horizontal synchronization pulse, and for 1 msec from the beginning of the vertical synchronization pulse in order to make the flyback of the electron beam invisible. The signal received by the monitor therefore contains 3 components: Video-Blanking-Synchronization-signal (VBS-signal).

According to the CCIR-norm, 1 line is scanned in 64 µsec so that the whole-image consisting of 625 lines is presented in 40 msec. The whole-image is divided into 2 successive half-images, each consisting of 312.5 lines and being displayed in 20 msec. The consecutive parts of the whole-image are interlaced, i.e. the lines of the first half-image are positioned exactly between the lines of the second half-image. At the border of the image, a few lines are suppressed by blanking and synchronisation. Therefore, only 296 lines per half-image are available for further analysis.

Registration of the X, Y-coordinates. In this section, a description of a basic circuit is given which allows the X, Y-coordinates of the upper corner of the white stripe S to be recorded (figure 1). The distance of this corner from the upper border of the image (Y-coordinate) is determined by line k. In this line, and for the first time in this half-image, the scanning electron beam reaching the corner of the white stripe causes the video signal voltage to rise. The X-coordinate is measured by counting the pulses of a fast clock from the beginning of the line (i.e. from the leading edge of the horizontal synchronization pulse) until the video signal reaches a given threshold. For electronic realization of the registration method (figure 2, a) discriminator D2 (passive filter + transistor) transforms the vertical synchronization signal to a short pulse resetting counter ZY. The latter counts the reshaped horizontal synchronization pulses provided by discriminator D3. Additionally, each output pulse D3 resets