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RESEARCH NOTE

CONTROL OF EYE MOVEMENTS WHILE RECORDING FROM SINGLE UNITS IN THE PIGMENTED RAT

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Many investigators report recording from single units in the rodent visual pathway without neuromuscular relaxants to immobilize the eye. This is in contrast to the situation in the cat where it has been shown that neuromuscular blocking agents are required to reduce eye movements to a tolerable level (Cleland and Enroth-Cugell, 1966; Rodieck, Pettigrew, Nikara and Bishop, 1967). Attempts at controlling rodent eye movements without paralyzing agents have been varied. Brown and Rojas (1965), Partridge and Brown (1970) and Hughes (1971) report no eye stabilization methods. Siminoff, Schwassman and Kruger (1966) used no eye stabilization techniques, but remapped receptive fields to assure that the eye had not moved. Montero and his colleagues (1968, 1973) used a disc pressed against the eye to mechanically stabilize the eye and report no eye movements. Shaw, Yinon and Auerbach (1975) state that eye movements were rarely encountered using deep urethane anesthesia. In order to prevent eye movements in cases with lighter anesthesia, a ring was sewn or firmly pressed around the eye and securely fixed to the apparatus. Wiesenfeld and Kornel (1975) pressed an eye ring against the eye and by projecting the optic disc once an hour over 6 hr, report finding only 1° movement. Unlike the previous investigators who used deep urethane anesthesia (IP dose of 1200–1700 mg/kg), Dräger (1975) working on the mouse rather than the rat, used light pentobarbital anesthesia exclusively (60 mg/kg initially and 0.2 mg as needed, IP) and no mechanical stabilization. She tried carefully to assess the extent of eye movements. By tracking light reflected from a small mirror placed on the corneal surface, she found slow drifts of up to 15° in 3 hr; but by locating the projection of the optic disc, she finds slow drifts of no more than 2 to 3 deg/hr which were within the tolerance necessary for her experiments. Dräger thus speculates that large slow drifts may be an artifact caused by glue irritation or the weight of the mirror. However, Dräger and Hubel (1976) report that if tectal coordinates were remapped 3–5 hr after the original mapping, a general shift of the projection of up to 15° in one direction was found. Although Dräger and Hubel believe swelling of tissue is the most likely cause for the shift, as they also point out, eye movements can well account for the observation. Dräger also attempted the use of Flaxedil, a neuromuscular blocking agent. Without careful regulation of the respiration volume, she found unresponsive or epileptiform discharges from the cortex, and thus she abandoned the use of Flaxedil.

We report here that in the rat, even under deep urethane anesthesia, there are large eye movements which are not eliminated by the mechanical means of cutting the conjunctiva and sewing on an anchored full eye ring. Neuromuscular blocking agents are required to reduce the movements to a tolerable level. In addition, we show that with well-regulated mechanical respiration, optic tract unit responses are normal.

Pigmented rats (Long–Evans) were initially anesthetized with urethane (1200 mg/kg) intraperitoneally with subsequent small doses as needed. A tracheal cannula was inserted. Blood pressure was monitored via a cannulation of the right carotid artery. Drugs and dextrose, when used, were administered by continuous infusion through a cannula in the left femoral vein. The rat was placed in a Baltimore Instruments stereotaxic apparatus. The upper eyelid of the left eye was removed and the conjunctiva was severed just behind its attachment to the globe. A full eye ring anchored to the stereotaxic apparatus was sewn to the conjunctiva on the side attached to the globe using silk sutures. A small chip of thin mirror, roughly 1 mm^2 in area and 1.5 mg in weight was attached to the cornea with cyanoacrylate adhesive (Permabond 102, Pearl Chemical, North Miami Beach, Fla.). A laser beam was reflected from the mirror onto a tangent screen 40 cm away. The position of the small laser spot was tracked and recorded as frequently as every 5 min. We had a sensitive measure of eye movements since the distance from eye to screen was great.

In one group of animals, no neuromuscular blocking agents were used. A typical result is shown at the top of Fig. 1. The elapsed time between each consecutive pair of labeled points is 30 min. In the first half hour, eye movements were recorded every 5 min; in the second half hour, every 15 min. Close monitoring of eye movements is important. If movements had been tracked less closely, say once every hour, then in this example only points a and c would have been recorded. An underestimate of eye movements would have been made. Large, slow drifts in one direction as shown in this record were typical. Also typical were patterns of movement which included large drifts and intervening periods of up to an hour with very little motion. Under this condition, we have recorded slow drifts of up to 4.5 deg/hr.

With the same preparation we now infused a solution similar to that used in cat (e.g. Rodieck, Pettigrew, Bishop and Nikara, 1967; Enroth-Cugell and

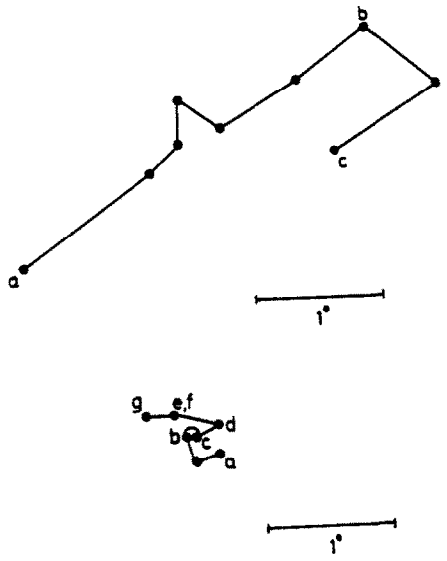

Fig. 1. At the top is shown a typical plot of eye movements recorded during 1 hr with only urethane anesthesia and a full eye ring sewn to the conjunctiva which is attached to the globe. The elapsed time between consecutively labeled points is 30 min. In the first half hour, eye movements were recorded every 5 min, in the second, every 15 min. At the bottom is a plot of eye movements over 3 hr when, in addition, continuous slow infusion of Flaxedil in combination with tubocurarine chloride and urethane was used. The elapsed time between consecutively labeled points is 30 min. Eye movements were checked every 5 min, but these were so small that they are not recorded here.

Pinto, 1970, 1972; Cleland, Dubin and Levick, 1971) of 10 mg/kg/hr of gallamine triethiodide (Flaxedil), 0.67 mg/kg/hr of tubocurarine chloride, and 30 mg/kg/hr of urethane after a loading dose of 5 mg/kg gallamine triethiodide and 0.3 mg/kg tubocurarine chloride, and the animal was mechanically respirated. The result was as pictured in the bottom half of Fig. 1. Again, the elapsed time between consecutively labeled points is 30 min. The position of the beam was checked every 5 min, but there was so little movement that these are not recorded. Over 3 hr time there was only a half degree of movement. It is clear from this record showing very little movement of the eye burdened with the weight of the mirror that the attached mirror cannot by itself artifactually cause the large, slow drifts that we see without neuromuscular blocking agents.

We next checked that this dosage of neuromuscular blocking agents did not adversely affect unit discharges from the rat optic tract. Recordings were made using a tungsten-wire-in-glass electrode (Levick, 1972) lowered into the optic tract. Details of the recording procedure are given elsewhere (Green, Tong and Cicerone, 1977). Figure 2 shows that the response of the same unit before and after administration of neuromuscular blocking agents is stable. This brief demonstration shows for rat what Enroth-Cugell and Pinto (1970) have already shown in the cat. That is, gallamine triethiodide doses in these ranges do not affect retinal ganglion cell responses. Unless an oxygen-rich gas mixture (up to 100%) was used, the ani-

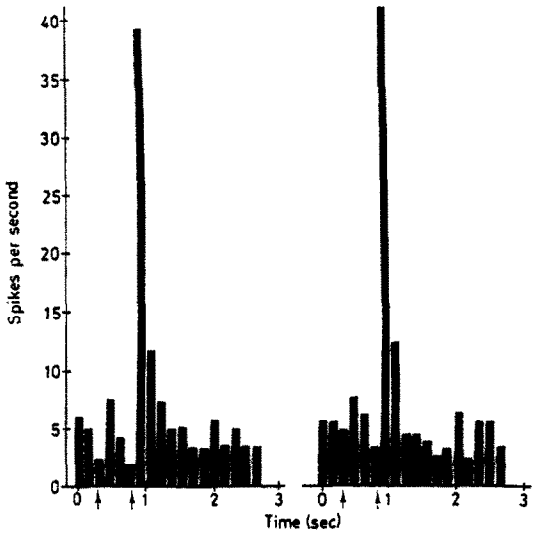

Fig. 2. The patterns of discharges from an OFF unit before and after administration of neuromuscular blocking agents were compared. The time histogram on the left is the unit's response to a full field stimulus about 1 log unit above dark-adapted threshold under urethane anesthesia only. Arrows mark the onset and offset of the stimulus. The record on the right for the same stimulus was taken 15 min after the animal had been paralyzed with gallamine triethiodide and tubocurarine chloride and was being maintained with mechanical respiration of an oxygen-rich mixture. The records are indistinguishable.

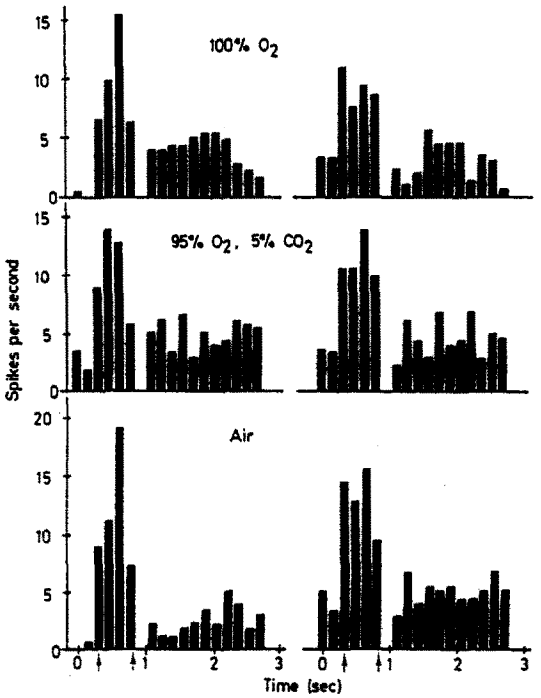

Fig. 3. After urethane anesthesia and during continuous slow infusion of Flaxedil and tubocurarine chloride, unit discharges were checked. The responses of an ON unit to a full field stimulus about 0.5 log units above dark-adapted threshold after 30 min exposure to 100% O_2, 95% O_2 and 5% CO_2, and air at the rate of 200 cm^3/min, 50 strokes/min, are shown. Two time histograms are shown for each condition. The variability between conditions is no greater than within any condition.

mal could not be consistently maintained for the long periods of time needed to conduct a successful single unit experiment. Recently we have been regularly using 50% air, 47.5% O_2, and 2.5% CO_2 and having animals survive for up to 36 hr. Therefore, we checked that high O_2 ventilation did not affect unit discharges. We varied the oxygen content of the ventilating gas by pumping air, 95% O_2 and 5% CO_2, or 100% O_2 at the rate of 200 cm^3/min, 50 strokes/min. Figure 3 shows the response of an ON unit after 30 min exposure to each gas composition. Two time histograms are shown for each condition. The variability between conditions is no greater than within any condition.

We have also tracked eye movements with urethane anesthesia and no mechanical stabilization. This proved to be unsatisfactory. Depending on the amount of urethane administered and the animal, there was more or less movement of the eyes. However, movements as large as 26 deg/hr were tracked.

If large movements of up to 4.5 deg/hr can be tolerated in certain experiments, then anesthesia in combination with mechanical stabilization is sufficient, However, for restriction of eye movements to 0.25 deg or less per hour, neuromuscular blocking agents, gallamine triethiodide in combination with tubocurarine chloride, were found necessary.

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