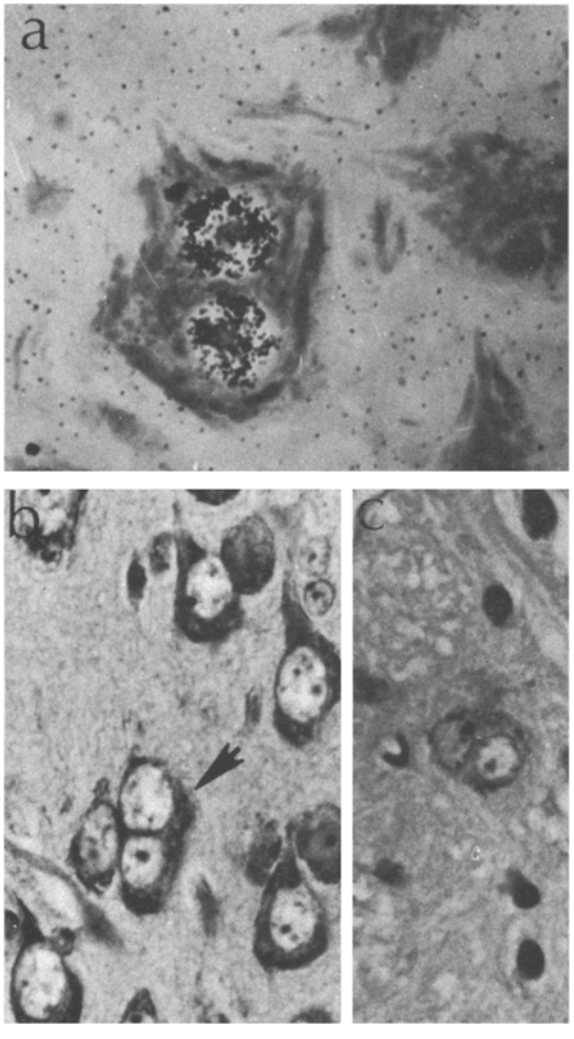
Binucleated neurons in the central nervous system of the laboratory animals[1]

G. D. Das

Department of Biological Sciences, Purdue University, West Lafayette (Indiana 47907, USA), 14 May 1976

Summary. Binucleated neurons were studied in the central nervous system of the rat and the rabbit. They were present in the young as well as the adult animals. The animals injected with thymidine-H^3 during their embryonic development showed labelled binucleated neurons. It is suggested that the neurons become binucleated during neuroembryogenesis, and differentiate into normal neurons morphologically and physiologically.

The binucleated neurons in the central nervous system have been observed by many investigators, but those in the sympathetic[2,3] and spinal[4] ganglia of the peripheral nervous system seem to have attracted far greater attention. Sosa and Savio de Sosa[4] have provided extensive literature survey on this problem, and have offered evidence on their existence in the spinal and Gasserian ganglia, spinal cord, mesencephalic nucleus of the trigeminal nerve and cerebellum of various species of mammals.



a A fully differentiated labelled binucleated motor neuron in the thoracic region of the spinal cord of a 6-week-old rabbit. This animal received thymidine-H^3 on day 11 of its embryogenesis, and on this day this neuron was formed. *b* A partially differentiated binucleated pyramidal cell (arrow) in layer V of the neocortex of a 15-day-old rat. Note a uninucleated neuron tightly juxtaposed to it. *c* A well-differentiated small-medium sized binucleated neuron in the midbrain reticular formation of a 30-day-old rat. ×460.

All the studies on this problem are based upon Nissl-or silver-stained material. In our thymidine-H^3 autoradiographic material on neurogenesis some observations were made on the binucleated neurons, and they are reported here.

Materials and methods. Laboratory-bred Purdue-Dutch rabbits were bred, and the day of breeding was taken as day 0 of gestation. One animal on each of the following days of gestation was anesthetized with Nembutal and prepared for laparotomy: days 10, 11, 12, 13, 14, 15, 16, 17 and 18. The uterus horns containing embryos were exposed, and thymidine-H^3 was injected into the amniotic cavity of each embryo individually with a Hamilton syringe (specific activity: 7.6 Ci/mM, 1 mCi dissolved in 1 ml of isotonic saline). The 10-day-old embryos received 8 μCi of thymidine-H^3, and the rest received increasingly larger amount of the radiochemical with an increment of 2 μCi for each day of increase in gestation. The embryos were allowed to develop to full term. 6 weeks after their birth they were anesthetized and perfused with 10% neutral formalin. The brains obtained from them were processed in the standard manner for histology and cut at 10 μm thickness. Every 10th section was saved. This material was then processed for autoradiography using Kodak-NTB-3 nuclear emulsion employing dipping technique. The autoradiograms were developed after an exposure period of 10 weeks and poststained with cresyl-violet. In addition to the material from the rabbits, a number of slides of serial sections obtained from the brains of 6-, 15-, 21- and 30-day-old rats were analyzed. These slides were stained with cresyl-violet.

Results. In the autoradiograms obtained from the rabbit brains binucleated neurons were observed in the cerebral cortex, basal ganglia, diencephalon, midbrain, cerebellum, hindbrain and spinal cord. Generally speaking they were large or medium in size. Very rarely small-sized neurons were binucleated. In this material most of the binucleated neurons had both the nuclei equally labelled (figure, a). Some neurons showed only 1 nucleus labelled and the other unlabelled. In such instances the labelled nucleus was found to be close to the emulsion surface of the section and the unlabelled nucleus to the bottom surface. Possibly this distance contributed to the apparent lack of label on the latter nuclei.

Analysis of material from the rat brains also showed the presence of binucleated neurons in various regions of the central nervous system including cerebral cortex (figure, b) and midbrain reticular formation (figure, c). Although no systematic attempt was made to quantify the binucleated neurons, they were rather few and scattered randomly all over the brain regardless of the age of the animal. It

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was estimated that there were at best 15–20 clearly identifiable binucleated neurons in a brain.

Discussion. The significance of the binucleated neurons has been viewed differently by researchers from different fields of investigations. Neuropathologists, in general, consider them to be the result of pathological condition of the brain[5–9]. Our findings as well as those of other workers on the presence of the binucleated neurons in normal brains do not support this viewpoint. Spiegel and Adolf[10], and De Castro[11] observed them in the sympathetic ganglia of the young, and suggested that they become binucleated during their genesis and retain the capacity to divide later during adulthood without further nuclear changes. Sosa and Savio de Sosa[12] after having examined material from 6 different mammalian species have concluded that such neurons represent amitotic division of nerve cells. Their observations seem to imply that fully differentiated neurons in normal adult brain are capable of dividing by amitotic division. Our findings on the labelled binucleated neurons suggest that in all probability binucleation of neurons is the case of complete nuclear division but incomplete cytoplasmic division in the precursors of neurons during neuroembryogenesis. The fact that they can continue to remain binucleated was supported by the findings made on the brains of the neonate rats and adult rabbits, and that they can differentiate as normally as uninucleated nerve cells was established by the differentiated cytology of these neurons in the adult brains. Although our observations do not deny or contradict the latter 2 viewpoints, they offer suggestions that the differentiated binucleated neurons may remain as such without ever giving rise to 2 uninucleated neurons, and that they may be as normal, both morphologically and physiologically, as other uninucleated nerve cells of the central nervous system.

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Hypoxia as a negative reinforcing stimulus in the squirrel monkey

P. P. Zwirner and U. H. Jäggi

Department of Pharmacology, Biological and Medical Research Division, Sandoz Ltd., CH–4002 Basel (Switzerland),
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Summary. Squirrel monkeys learned to avoid hypoxia by pressing a bar. Each bar-press replaced the noxious gas mixture with normal air for 3 sec. The data further indicated that O_2 itself has no positive reinforcing effect.

Squirrel monkeys (Saimiri sciureus) have been extensively and successfully used in a large number of operant conditioning studies, using a variety of different schedules. These animals learn to respond to both positive and negative stimuli[1]. Positive reinforcing stimuli have included food, water, light[2] and social contact[3]. The most frequently employed negative reinforcing stimulus is electric shock[4], but little is known about the effects of other noxious stimuli, such as exposure to an environment made unpleasant by change in temperature or atmospheric variations.

The vulnerability of the nervous system to lack of oxygen[5] and the impairment of performance in hypoxia[6] have been well documented. We have, therefore, investigated whether squirrel monkeys could learn to detect hypoxia and attempt to alter the environment when given the opportunity.

Methods. 6 squirrel monkeys with previous experience in conditioned avoidance experiments were used. The subjects were seated in a restraining chair and fitted with an airtight helmet which was perfused with a gas mixture containing known concentrations of oxygen and nitrogen. The experiments were carried out in a sound proof cage equipped with a pressing bar. The program was arranged so that each bar-press led to replacement of the gas mixture with normal air for a period of 3 sec. The oxygen content of the perfusing mixture was continuously monitored using a Beckman oxygen meter.

In an initial training period, the animals were exposed to a gas mixture containing 9% oxygen and, under this condition, each bar-press increased the oxygen concentration by 4%. All the animals learned to press the bar within 1 h, and after 2 weeks with daily 1-h-sessions, the number of bar-presses had stabilized in 4 monkeys. Each of these 4 animals was then tested with gas mixture

Number of bar-presses during 1 h under defined oxygen concentrations

% O_2	Animals s 46	s 101	s 70	s 65	Mean	Change (%)
9	922	719	1494	1651	1196	0
11	616	455	211	1137	604	–49.5
13	593	263	502	1078	609	–49.1
15	343	190	156	1209	474	–60.3
17	335	271	147	984	434	–63.7
19	321	209	123	627	320	–73.3
21	391	178	100	434	275	–76.9

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