into account that the K^+-electrode potential was practically uncontaminated by the negligible field potential, the above value could be interpreted as an E_K-potential corresponding to a $[K^+]_e$-increase from 3.0 mM to 3.31 $\pm$ 0.04 mM. The average duration of the $[K^+]_e$-increase was 37 sec and was correlated with the duration of EEG desynchronisation. The maximum $[K^+]_e$ was attained 13 $\pm$ 2 sec after desynchronisation onset.

The above results confirm the feasibility of $[K^+]_e$-recording in unrestrained rats. Although in the present study the movement was limited by light anesthesia and recording in freely moving animals was severely disturbed by artefacts, it is conceivable that further improvement of the technique may produce supression of capacitative and electrostatic interference and make it possible to record $[K^+]_e$-changes in behaving animals.

The amplitude of E_K is several times higher than the corresponding field potential changes[13]. The $[K^+]_e$-changes seem to be much more sensitive and straightforward index of sustained activity of large neuronal populations than the corresponding slow potential changes. It can be expected that K^+-electrodes will provide an important method in tracing neural circuits participating in various behavioral processes.

13 H. Caspers, Proc. int. Union Physiol. Sci, XXII Int. Congress, Leiden, vol. I, part I, p. 442 (1962).

Effects of hypergravity on rat fertility, pregnancy, parturition and survival

J. Ishay and L. Barr-Nea

Department of Physiology and Pharmacology and Department of Histology and Cell Biology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv (Israel), 2 June 1976

Summary. Persistent centrifugation (at 1.18 $\times$g to 1.47 $\times$g) of pregnant rats reduced the number of deliveries and the survival time of the newborns. The extent of the damage increased with increasing distance from the axis. Male fertility was reduced.

Introduction. In previous papers various pathological changes have been reported to occur in animals kept under persistent centrifugation[1-6]. In the present study we describe the effects of centrifugation on fertility, pregnancy, delivery and survival of rats under persistent centrifugation at relatively low additional g-forces.

Materials and methods. A special four-armed centrifuge was designed and constructed to accomodate the regular rat breeding cages (BC). 5 such cages were attached firmly to each arm at different distances from the center of rotation. The resultant forces which acted upon the cages when the centrifuge rotated at 25 rpm, were as follows: first cage at 86 cm from the center – 1.27 $\times$g, second cage at 104.5 cm from the center – 1.34 $\times$g, third cage at 125.0 cm – 1.41 $\times$g, fourth cage at 141.5 cm – 1.43 $\times$g, and fifth (terminal) cage at 150.0 cm – 1.47 $\times$g. Concommitantly 2 controls were used: a) a rotating control of 2 BC's placed in opposition at a distance of only 20 cm of the center of the centrifuge (resultant g-force 1.04); b) a stationary control of 6 BC's placed on the floor behind the centrifuge (figure 1).

Once daily, but at different times[7], the centrifuge was stopped for 15 min in order to check the animals, supply them food and water, clean the cages and service the machine.

Test design. The centrifuge was activated within a special room, under fluorescent illumination and at constant temperature of 26 °C. Each animal was spun for 4 weeks,

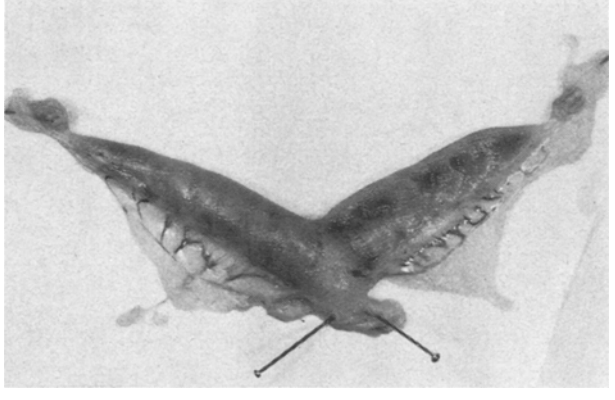
Effects of increasing gravitational force on the reproduction pattern of adult rats and survival of the offspring

Cage No.	g	Females placed in centrifuge at mid-pregnancy (day 10–13)			Females placed in centrifuge at the beginning of pregnancy (2–4 days after confirmed mating)		
	Resultant forces	Number of normal deliveries	Number of*** live newborns (per litter)	Live span of newborns in days*** (per litter)	Number of normal deliveries	Number of*** live newborns (per litter)	Life span of newborns in days
Center (rotating control)	1.04	5	8, 8, 11, 9, 8 (9)	normal (more than 60 days)	5	10, 11, 7, 8, 9 (9)	normal
1	1.27	5	8, 8, 9, 7, 10 (8)	normal	5	9, 10, 7, 8, 8 (8)	normal
2	1.34	5	10, 7, 9, 7, 8 (8)	normal	5	8/9, 9, 7, 6, 5/6 (7)	normal
3	1.41	5	8, 11/12****, 8/9, 10, 7/9 (9)	4 groups normal last litter = 1 day	5	6/8, 5/8, 7, 8/9, 7 (6.5)	normal
4	1.43	4/5*	6/8, 8, 8/9, 8/11 (7.5)	4, 3, 6, 10 (6)	3/5	8/9, 5/7, 4/7 (6)	normal
5	1.47	3/5**	7/12, 3/7, 5/7 (5)	2, 1, 2 (2)	1/5	4	3.5 days

* 1 abortion, ** abortions, *** mean in brackets, **** ratio of live newborns/total litter. The delivery rate and life span of rats in stationary control BC's was normal for that breed.

Fig. 1. Picture of the four-armed centrifuge. Each arm holds 5 rat breeding cages. 2 rotating control cages stand in the center of the centrifuge. Behind the centrifuge 2 cages of stationary control animals.

a



b

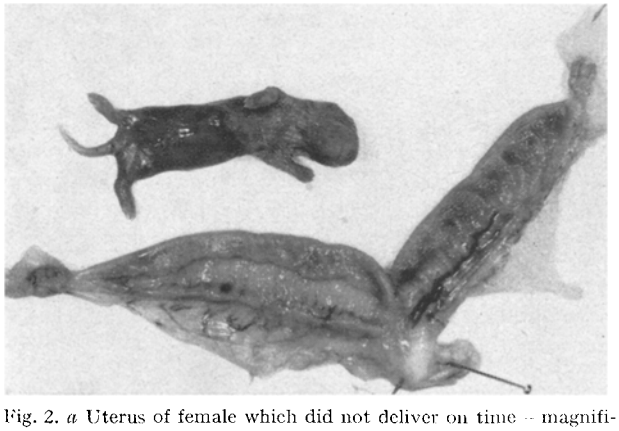


Fig. 2. *a* Uterus of female which did not deliver on time – magnification × 2. *b* Same uterus open – one dead embryo was found in the right horn and blood clots in the left one.

an interval of time sufficient for it to accomodate to the centrifuge, to mate, to deliver or to abort. BC's containing newborn rats were kept for longer periods in the centrifuge in order to observe the survival of the neonates under centrifugation.

Pregnancy, delivery and survival. The success of pregnancy and delivery under centrifugation was assessed on: a) females introduced into the centrifuge as 2–4 days of pregnancy, and b) females introduced at day 10–13 of pregnancy. Conception was confirmed by the presence of spermatozoa in the vaginal smear and this was counted as day O of pregnancy. Each test group comprised 25 pregnant females: 5 × females × 5 positions along the centrifuge arm. Additionally, 5 pregnant females were placed in the pivotal BC's as rotating controls and some outside the centrifuge as stationary control. The pregnant females were examined daily for possible signs of abortion. Newborn rats were counted at delivery, examined daily thereafter and their life-span recorded. Females that did not give birth at the calculated time were killed and necropsised 2 days later, their ovaries and uteri examined for evidence of abortion and the number of fetuses, if any, compared to the number of corpora lutea.

Fertility. Only rats of ascertained fertility were used (i.e. after successful copulations for the males and one

1 M. G. Prives, Moscow Med. Press *3* (1972).
2 A. S. Pankova, Space Biol. Med. *6*, 84 (1972).
3 P. L. Sannes and T. G. Hayes, Avi. Sp. Env. Med. *46*, 780 (1975).
4 E. Up. Chae, Aviat. Space Env. Med. *46*, 703 (1975).
5 H. L. Stone, H. H. Erickson and H. Sandler, Aeronaut. Med. *45*, 498 (1974).
6 F. Altman, Aeronaut. Med. *44*, 418 (1973).
7 C. C. Wunder, in: Gravity and the Organism, p. 389–415. Ed. S. A. Gordon and M. J. Cohen. Univ. of Chicago Press, Chicago 1971.

successful pregnancy for the females). In all 20 couples were tested: 1 (couple) × 5 (positions) × 4 (arms of the centrifuge). After a spinning period of 4 weeks, the fertility of both males and females was checked anew in stationary cages for 4 weeks.

Results. Results on rat parturition and newborn longevity under persitent centrifugation are summarized in the table. As can be seen from the table, females in cages (positions) 4 and 5 of the centrifuge showed a tendency to abort. This tendency was enhanced in females which were spun at the beginning of their pregnancy. The life of the newborns in these 2 cages was significantly shorter than in the others ($p < 0.005$). In contrast, the life span of the adult males and females was apparently not affected by spinning. The females in BC's 4 and 5 showed poor mammary development, but otherwise displayed normal maternal behaviour. Of the 12 couples placed along 2 arms of the centrifuge (10 tests and 2 rotating controls), only one couple (in cage 3) did mate successfully. The couples were removed from the centrifuge after 3 weeks of spinning; of the spun males, 6 were fertile immediately after removal, 3 were not able to fertilize any female for at least 3 months, and 3 remained unfertile for one month but were then able to impregnate females which gave birth to normal youngs. All the spun females proved fertile after removal from the centrifuge, giving birth to normal youngs with no delay.

Figure 2 shows the uterus of a female which did not give birth in time and was necropsized. The right horn was swollen and the left one was empty (a); after opening the uterus (b) one dead embryo was found in the right horn, while blood clots were found in the left horn. Most females which did not deliver at the right time presented a similar pathological picture.

Discussion. This paper deals with the effects on rats of persistent centrifugation at a relatively low additional gravitational force of 1–1.47 × g. Under these conditions, longevity of adult rats was not apparently affected, but that of the newborns was significantly shortened at a resultant gravitational force exceeding 1.41 × g.

It seems that with a g-force higher than this critical one (in cages 4 and 5) the success of pregnancy depends on the time at which the pregnant rats start spinning: the earlier it has started the less successful the pregnancy. In cases where delivery has been delayed beyond the calculated time, necropsy shows various degrees of hemorrhage and damage to the fetus. All such fetuses are dead, in some cases their sizes indicating that development has stopped at mid-term, and in others that they have reached full-term, but for some inknown reason delivery has not taken place. In general there is no correlation between the spinning time of the pregnant rat and the time of abortion; in most cases the only macroscopical findings are blood clots in the lumen of the uterus and contraction of the myometrium. In cases of abortion, the ovaries contain corpora lutea at various stages of regression. However, no pathological changes have been observed in brain, muscles and viscera, as reported earlier to occur under persistent centrifugation[1–6]. It is thus possible that the reason is the relatively low additional gravity force used in this study.

We do not know whether the failure to deliver is in any way related to the feeding state of the animals. During the brief intervals in which the centrifuge was stopped, all the rats seemed quite normal and ate and drank as usual, but whether they did so during the spinning could not be determined. As mentioned earlier, the mammary glands and teats of the nursing females were poorly developed and their short-lived offsprings appeared starved and emaciated. At this stage of study it is difficult to say whether this mammary insufficient development was due to a) undernourishment of the female rat, b) stress of centrifugation on the female, c) failure of the offsprings to suckle due to the physical stress of centrifugation, i.e. inability of the neonates to fasten on to and to hold the mother's teat under the added gravity. (Such failure to suckle would rapidly provoke cessation of lactation in the mother.) It is of course possible that several of the abovementioned reasons combine to contribute to the early death of the offspring in cages 4 and 5. In contrast, the neonates in cages 1–3 and in the rotating control cages develop quite normally, although they were slightly underweight compared to neonates in the stationary control cages.

Effects of monovalent cations on the responses of motoneurones to different groups of amino acid excitants in frog and rat spinal cord

R. H. Evans, A. A. Francis and J. C. Watkins[1]

Department of Pharmacology, The Medical School, University of Bristol, University Walk, Bristol BS8 1TD (England),
29 July 1976

Summary. Classification of excitatory amino acids into different groups, of possible value for transmitter identification, can be made on the basis of the differential effects of altered external [Na^+] and [K^+] on motoneurone depolarization in frog and immature rat spinal cord.

It is generally considered that the depolarization of mammalian central neurones by L-glutamate and related amino acid excitants involves an increase in the permeability of the neuronal membrane to Na^+ ions[2–6]. However, the structures of excitatory amino acids show considerable diversity[7,11], and it is not certain that the mechanism of their actions is similar in all cases. Indeed, Engberg et al.[8] have recently demonstrated that the excitatory action of DL-homocysteate on spinal motoneurones is associated with a decrease rather than an increase in membrane conductance. We now describe experiments conducted in vitro on the spinal cord of the frog and immature rat in which amino acid-induced depolarization of motoneurones, recorded from ventral roots, was studied in superfusion media of varying ionic composition. It was found that the responses of different amino acids were affected differently by the ionic changes studied, and that amino acids could be classified within distinct groups on the basis of the results obtained. Some of the observations suggest the possibility of more than