A METHOD OF PROLONGED SLEEP DEPRIVATION FOR SMALL ANIMALS

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Experimental sleep deprivation is one of the few methods of reproduction of a state of overstrain, followed by nervous exhaustion. If it continues for long enough, the experimental animal invariably dies.

In most investigations procedures compelling the animal to perform a particular type of muscular work are used to maintain a prolonged state of wakefulness [1, 2, 5, 6, 8, 9].

In 1962 a full account was published of an apparatus for producing prolonged sleep deprivation in mice [3]. The same principle has been used in the construction of an apparatus for working with rats, but certain improvements have been incorporated (Fig. 1).

This apparatus consists of a cylinder 1.25 m long and with a base diameter of 50 cm, subdivided by metal disks (1) into 5 compartments. The cylinder is made of metal gauze with a 2 mm mesh. Each compartment, which is in effect a cage, has a door (2). The metal axle (3) of the cylinder is fixed to both ends, and the ends of the axle pass through bearings (4) mounted in upright supports on a special table. The motor (5) drives the axle through a reducing gear (6) and belt. The cylinder is revolved continuously day and night at a constant speed of 2.3 rpm. Throughout the 24 h the rats in the cylinder move through a maximal distance of 5.2 km. According to Munn [7], albino rats if kept at liberty run between 1.8 and 27 km or even more each day. Accordingly, in the first days of the experiment the animals are forced to cover a distance close to the minimal distance they would cover spontaneously, and thereafter their motor activity becomes still further reduced. In experiments performed by different workers, the speed of the cylinder varied from 1.1 to 4.8 rpm. The motor activity of the rats in this apparatus is also limited by the fact that their hind limbs are first bound with adhesive plaster to prevent trauma from the metal gauze. A very important detail of the apparatus introduced into the authors' modification consists of 4 wooden flanges (7), 2.5-3 cm high, in each compartment. The significance of these will be described below.

Two animals can be kept in each compartment. They feed during the experiment on concentrated food prepared by Mosoblsovnarkhoz enterprises (Burtsevskii Alcohol Factory) and containing 12 ingredients. The food pellets are placed on the floor of the compartments, they rattle when the cylinder turns,

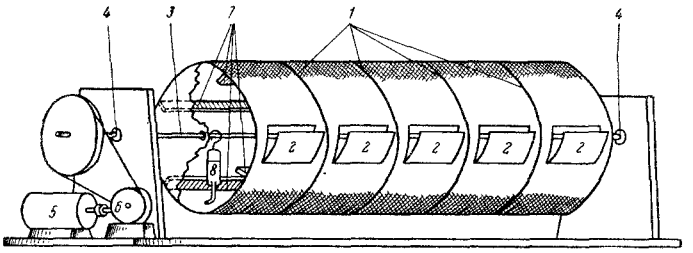

Fig. 1. Scheme of apparatus for producing prolonged sleep deprivation in rats. 1) Metal disks dividing cylinder into cell compartments; 2) doors; 3 axle; 4) bearings; 5) motor; 6) reducing gear; 7) flanges inside each compartment; 8) water container hanging from axle.

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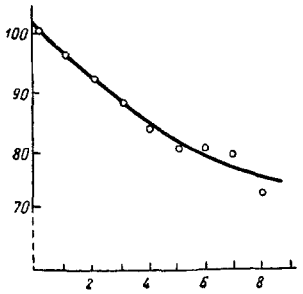

Fig. 2. Changes in body weight of rats in relation to duration of sleep deprivation (mean of 13 observations). Abscissa) days of experiment; ordinate) body weight (in %).

and this is another factor preventing the rats from sleeping. Animals, accustomed for a few days before the experiment to this food, will eat it readily when the apparatus is in motion. Of vegetables a little carrot may be given. Water is supplied from a container (8) hanging from the axle and consisting of an inverted glass cylinder fitted with a curved spout from which the animals may drink, also while the cylinder is in motion. For a few days before the experiment begins the rats must be trained to use this water container. Although for the first 2-3 days without sleep the rats eat and drink by their own efforts, as they become more fatigued the cylinder has to be stopped 3-4 times a day for a few minutes and the animals made to drink by applying the spout of the container to their mouth.

During the first 2 days without sleep the animals usually appear alert. If their hind limbs are not bound with adhesive plaster, at first they are very active, clinging to the gauze, jumping, climbing on to the axle, and injuring their feet. Binding the paws not only prevents trauma, but by restricting mobility, it also prevents the animals from climbing on to the axle, on which they could balance, rest, and even sleep if their paws were free. With increasing fatigue of the nervous system the animals become less mobile and finally they lie on the bottom of the compartment and sleep, paying no attention to the rotation, and sliding on their back or side over the moving gauze. However, this is observed only if no flanges are fitted. This was how the mice [3] and rabbits [2] behaved in these conditions in other investigations.

After sleeping for 1.5-2 h in this way the rats again feel awake and start to move about, to eat, and to drink. To prevent this sleeping, which ruins the whole purpose of the model, the flanges were added: the very fatigued animals lying on the floor of the compartment were caught by the flanges and carried upward by them; then they fell off the flanges and either rolled over or clung on to the gauze with their paws. In either case, they awoke—lifted their head, opened their eyes, and walked a few paces. Each flange thus automatically awoke the fatigued animals which could not help going to sleep. Because each compartment contained 4 flanges, this forced awakening took place every 7-8 sec. Consequently, even if the extremely tired rats managed to rest after several days of sleep deprivation, it was only for a few seconds.

The flanges in the compartments are also useful because they enable the animal to reduce its muscular activity from the outset. Very soon after the beginning of the experiment, the rats adapt themselves to the new conditions and sit on the flanges. There they stay quietly until the rotation of the cylinder carries them too high, when they jump down on to the next flange as it comes up to meet them. As a result, the muscular activity of the rats during the 24 h period is evidently less than would be involved in running a distance of 5.2 km.

For each animal 20 g of concentrated food is provided daily in the compartments, and about 4 g spills out through the gauze as crumbs. With the development of fatigue more and more of the food remains uneaten and the rats lose weight. Whereas on the 1st day of the experiment the animals eat 15-16 g of food, on the 2nd day this amount falls to 12 g, and on the 3rd day to below 10 g. The results of observations on the loss of weight relative to the duration of sleep deprivation are shown in Fig. 2. Exhaustion of the nervous system, demonstrated by inability to maintain the normal position of the body and to overcome the urge to sleep developed in the animals on the 3rd-5th day, sometimes earlier or later. By this time (the 4th-5th day) the body weight of the rats had fallen by 19.4%, a value in agreement with the observations of other workers [9].

The results of 33 experiments showed that 36.4% of the animals died on the 4th day and 24.2% on the 5th day, so that 60% died on the 4th and 5th days together. On the 3rd day about 12% of the rats died, and on each of the 6th, 7th, and 8th days—about 9%. To study the animals in a state of extreme fatigue of the nervous system from sleep deprivation, it appears that the animals should be sacrificed after a stay of 4-5 days in the apparatus.

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