Decorticate Female Rats and Reproductive Functions

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ABSTRACT

Hooded rats were operated to achieve maximum removal of cortical tissue (decortication- DC). DC was achieved which was later confirmed by serial histology. The rats and well matched controls were studied as to their reproductive cyclicity using vaginal smear technique, and their ability to achieve functional oestrus and mating – including pregnancy. From histological demonstrations, two groups of animals emerged – DC with intact olfactory cortex and DC without olfactory cortex. Generally all DC rats did not cycle normally unless kept in a male cage. Olfactory DC rats did not cycle at all and did not become pregnant. Non-olfactory DC rats became pregnant when exposed to male and were able to carry pregnancy to term in varying degrees of success. It was concluded that decortication impairs reproductive function in the female rat and that this may be due to impairment of hypothalamic function. The possible role of local control of reproduction in DC rats is mentioned.

Keywords: Follicle stimulating hormone, Hippocampus, Olfactory, Ovulation.

INTRODUCTION

Evidence in support of higher cortical involvement in the control of the oestrous cycle in the rat is scanty. Donovan (1) discussed extensively the control of release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in relation to environmental influences via the olfactory and visual stimuli. Bruce (2) studied the effect of odors on the oestrous cycle in the female mice. Priedkalns (3) suggested that the oestrous cycle in the female rat can be stimulated by male odors and there are also several reports which suggest that female rats can inhibit their own cyclicity when placed together in the same cage.

Donovan (1) discusses the control of higher cortical centers on the oestrous cycle in the rat using models of rat hypothalamic de-afferentation. In such experiments, the hypothalamus was isolated from the pituitary stalk and other higher structures of the neuraxis by division of afferent fibers only. It is now well recognized that the hippocampus which is a limbic system structure and which is also part of the oldest cortical system – the archicortex (4) and the amygdala, which is a basal ganglia structure, have been identified to influence the cyclicity of the female rat directly through the cyclical hypothalamus. Stimulation of the hippocampus can lead to ovulation if this is done on the 2nd day of diestrus while electrical stimulation of the hippocampus has been known to cause ovulation (1). The nature of suprahypothalamic control of reproduction at least in the female is not very well understood.

Several investigators have attempted to achieve decortication in the rat in order to study various physiological phenomena. Decortication in other animals aside from the rat is not easy to maintain chronically but the rat seems to withstand the technique remarkably well and may be kept for over a period of 18 months (2-4). It is the purpose of this paper to

determine the effect of the removal of the cerebral cortex on the reproductive behavior of chronically decorticate hooded female rats. These include ability of the rats to become pregnant, carry the pregnancy successfully to term, deliver litters and suckle to maturity. Interest also include reproductive behavior such as ability to inhibit oestrus and other aspects of general behavior of the decorticate rat.

MATERIALS AND METHODS

Hooded rats were used for the study in view of their ability to withstand surgical procedures better than albino rats. They weighed between 100-250 g and were 40 in number and 5 control rats and 5 sham operated rats. The rats were decorticated by the open method in which brain tissue was removed under direct vision. Incision was made into skin overlying the sagittal suture and this was widened in order to expose the frontal and parietal bones. Holes were drilled into the frontal and parietal bones about 0.5 mm diameter using 16 gauge needle (Luer-lok). The flap of bone thus created, which did not reach the frontal piece overlying the olfactory cortex, was then carefully lifted medially in order to identify the underlying dura (Figure 1). Incision was made into the dura and cortical tissue exposed. Fine currette was then used to scoop cortical tissue under direct vision piecemeal until the corpus callosum was identified. Bleeding points were carefully arrested by diathermy or pressure mopping with surgical swab or both. This procedure was also repeated for the other side of the cranium. Flaps were lifted back into place after the procedures and skin sutured over the cranial flaps using silk sutures 2/0.

Since the open method of decortication was incapable of reaching the olfactory cortex (Fig. 1), it was necessary to destroy the olfactory cortex in half of the experimental animals (n=15) by stereotaxic method at coordinates -1.54 mm (anteroposterior), 0.3 mm (lateral) and 0.11mm (dorsoventral). Absolute alcohol was used as lesioning agent (0.03ml). The destruction of the cortices were later on confirmed by histology for the olfactory cortex and by visual method after extraction of the whole brain for the rest of the cortex. It was adjudged that up to 95% of cortical tissues were removed in all operated rats.

Two experimental groups of rats emerged from the above techniques; those decorticate rats with intact olfactory cortex (n=15) [and those without it (n=15).]

Animals were kept in separate cages after surgery and nursed warm at a temperature of about 35^{o}C. They were provided with food and water and libitum. They were examined daily and weighed for the first 14 days. They were examined for reflexes, muscle tone and power, ability to feed and take water, pain and other somaesthetic senses. Pain was tested by the use of forceps pressure on the tail which elicited cry if positive; touch and pressure by light or deep touch by the fingers when animal was not looking.

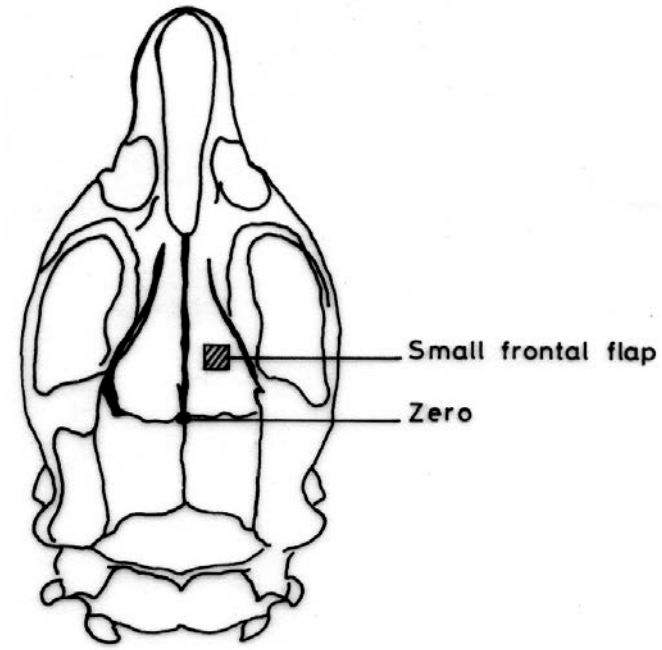

Fig. 1. Dorsal aspect of rat skull showing removal of large flap along the frontal bone for open decortication procedure. Zero coordinate for stereotaxic is shown.

Table 1: Cyclicity pattern as adjudged by vaginal smear cells in the same female rats as in Figs.(1, 3, 5, 6) together with two others (2, 4) after pairing with male in the cage.

Operative type	Day 1	2	3	4	5	6	7	8	9	10
1. Non-olfactory decorticate rat (preoperative weight 100g)	E	M	M	E	M	M	E	E	E	P
2. Non-olfactory decorticate rat (preoperative weight 125g)	D	D	D	P	P	P	D	D	P	D
3. Olfactory decorticate rat (preoperative weight 110g)	D	D	D	P	P	P	D	D	P	D
4. Olfactory decorticate rat; stereotaxic (preoperative weight 110g)	D	D	P	P	P	D	P	P	P	D
5. Control (preoperative weight 120g)	E	M	D	D	P	E	M	D	D	P
			1	2				1	2	
6. Sham operated (preoperative weight 125g)	M	D	D	P	E	M	D	D	P	E
		1	2				1	2		

Seven months post-operative, the animals were subjected to vaginal smear daily examinations for 15 days, (i.e. 3 cycles of 5 days each). Smears were taken by the use of glass pipettes containing normal saline which was placed in the vagina. The normal saline containing sucked vaginal secretion was then subjected to microscopic analysis after staining in 0.5% toluidine blue in borax and fixing in 70% alcohol. They were interpreted by the conventional cytological methods for identifying estrous cycle in the rat (8,9) as follows – predominantly leukocytes (98%) – diestrus; predominantly cornified epithelial cells – estrus; large amounts of epithelial cells with leukocytes – proestrus and considerable amounts of leukocytes – cornified epithelial cells and epithelial cells – metestrus.

The combination of diazepam (0.3mg/kg body weight) and ketamine (50mg/kg body weight) given intramuscularly was found useful for anesthesia in these rats during the procedures of decortication and stereotaxis.

RESULTS

Mortality rate of procedure was found to be 10% with 3 rats dying intra-operatively due to excessive bleeding. Hooded rats tended to withstand excessive surgery better than other forms of strains (2). All decorticate rats post-operatively exhibited the following:

1. Characteristic decorticate posture with all limbs placed under the abdominal

wall and the head close to the limbs in a compact position (Fig. 2).

2. Hyperexcitability as adjudged by sensitivity to touch, painful stimuli, noise, etc. The animals reacted by jumping – resembling the hare jump.
3. All animals did not feed at all or not properly for the first seven days. Some would attempt to feed on their fecal matter.
4. All animals recovered 4 weeks post-operative so that their feeding habit, ability to gain weight, general posture and behavior were no longer distinguishable from those of control animals.

Fig. 2. Compact position maintained by rats in immediate post-operative period.

Reproductive Cycle

The two animal groups exhibited differing cyclical behaviours. No operated rat cycled normally or spontaneously. Figure 3 shows cyclicity in olfactory decorticate (DC) and non-olfactory DC rats. Non-olfactory rats tended to cycle around oestrus and metestrus while olfactory rats cycled around diestrus and proestrus. These cyclicity patterns were obtained when the animals were placed in separate cages. Table 1 shows cyclicity after pairing the animals with normal male rats. The non olfactory DC rats immediately began to cycle and became pregnant at varying periods of time while the olfactory DC rats still did not cycle and also did not become pregnant. Sham operated and control rats however became pregnant. Postmortem examination of those olfactory DC rats showed corpus luteum in ovaries and sometimes swollen mammary glands.

Mating and pregnancy

The non-olfactory DC rats which became pregnant carried their pregnancies to term with varying degrees of success; some were able to have just 1 litter while others had up to 6. Some had stillbirth but no congenital abnormalities were observed. Those which had normal litters were unable to suckle and the litters died within a few days after delivery which they immediately cannibalized.

Decorticate procedure

Generally hooded strain tended to withstand surgery better than wistar and they did not suffer from profuse intraoperative bleeding as the wistar strain. It is possible to remove some parts of the caudate-putamen together with the supracallosal cortex as in this strain of rats without increase in mortality.

Ketamine anesthesia

All animals operated for decortication showed resistance to ketamine hydrochloride anesthesia post-operatively, even at a dose 10 times the normal; i.e., 120 mg/kg body weight or above.

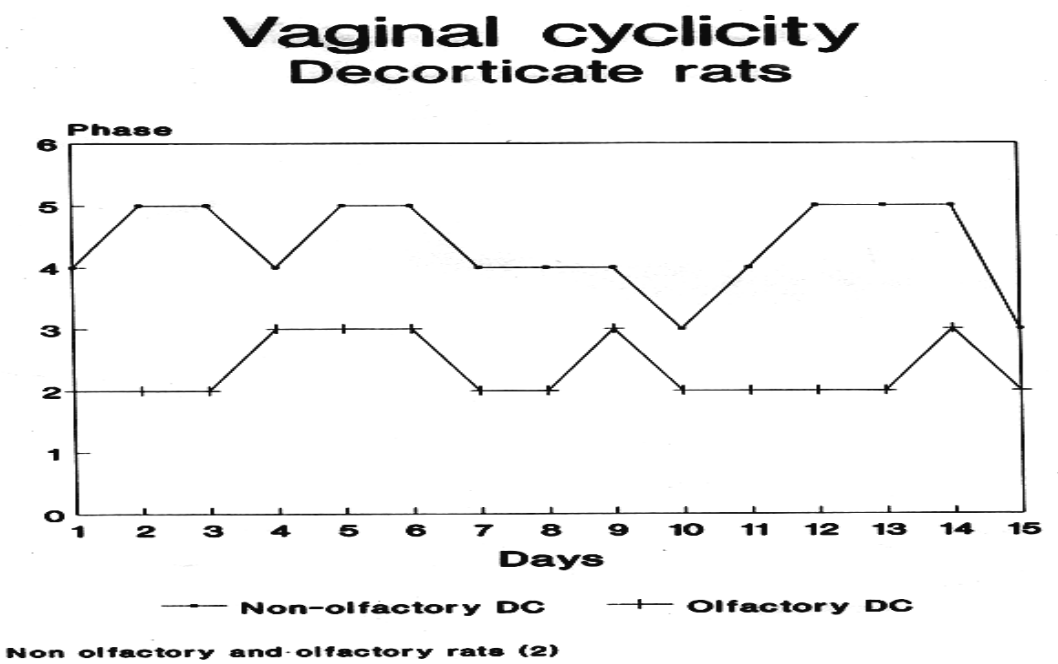

Fig. 3. Cyclicity pattern as adjudged by vaginal smear cells in 4 female rats kept in separate cages for 15 days. A. Decorticate rats (n=2); B. Control rats (n=2); 1-Diestrus, 2-diestrus II, 3-Proestrus, 4-estrus, 5-metestrus. Cyclicity was studied 7 months post-operative.

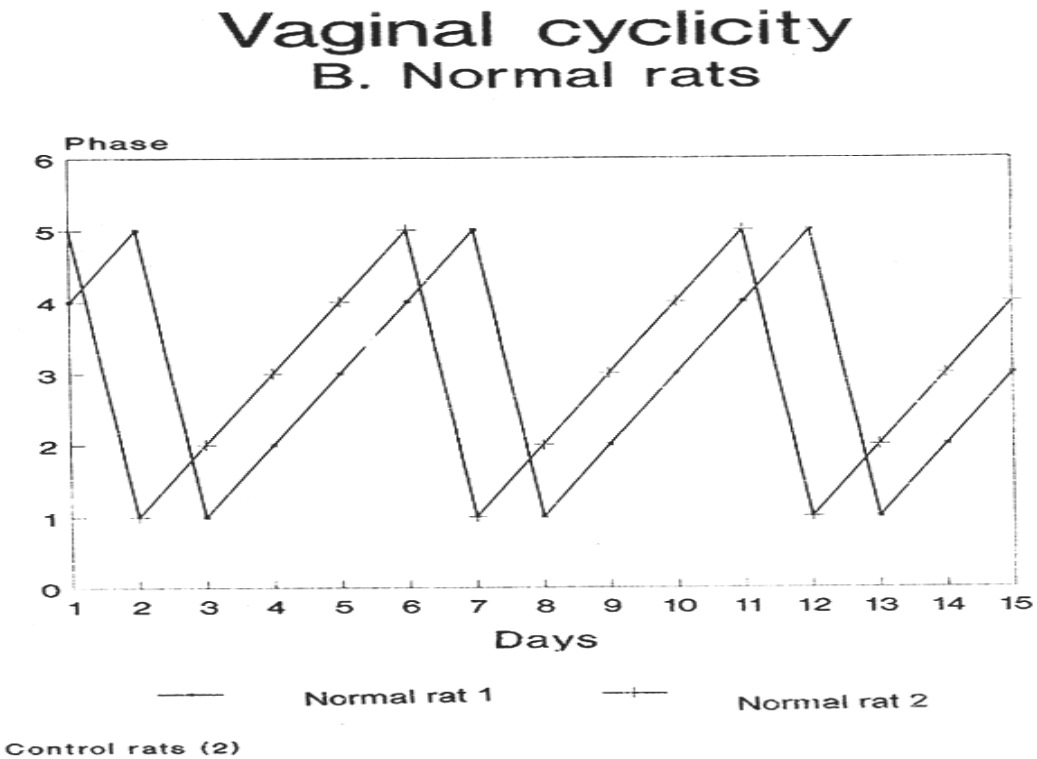

Fig. 4. Gross appearance of dorsal aspect of a non-olfactory decorticate rat brain 15 months after the procedure (B). A, C and D are sham-operated.

DISCUSSION

Hooded rats are quite capable of withstanding surgery including neurosurgical procedures better than albino rats and hence the extensive use in this experiment. Several animal species have been used for procuring decorticate animal preparations for physiological studies (5) but they tend to have low survival rates. Dogs may not last for more than one week (5) but the rat can be chronically kept decorticate and may therefore serve the purpose of testing the functions of the cortex in various systemic studies (6,7). It is remarkable that rats withstand the procedure so well and that even perinatal rats can survive and grow into adults as decorticate animals (6). It is already well known that the rat is most resistant to ketamine hydrochloride anaesthesia and this has been suggested to be as a result of lack of large functional neocortex in the rat (8) since there is predominant need for a functioning cerebral neocortex in order for this anesthetic agent to work at all (10-16). Hence remarkable recovery of rats after decortication suggest that subcortical tissues are capable of taking over most of the somaesthetic and motor functions of the rat in the absence of a functioning neocortex. Indeed, Deyo, Panksep and Abott (6) reported that perinatal decorticate rats can learn a spatial task using subcortical structures.

While other functions, especially musculo-skeletal, can go on without a functional neocortex in the rat, there is an obvious impairment of the functions of reproduction. Thus the female rat is unable to cycle normally and on its own in the absence of the cortex. It is already well known that exteroceptive factors aid cyclicity. What is not well recognized is the extent (1). Removal of such factors as in the DC rats may affect cyclicity adversely probably via effects on the suprahypothalamic structures such as the hippocampus. Such exteroceptive influences as visual, auditory and olfactory, tend to affect estrus cycle via the combined effect on the hypothalamus (1). Priedkaln (3) discussed at length the effects of visual, auditory and olfactory stimuli on implantation and general reproductive performance in many animals. Male odors are known to stimulate estrus cycle but inhibit conception. In the present experiment, those animals with intact olfactory cortex were capable of becoming pregnant while those without it were not. This demonstrates the effect of olfactory system on reproductive performance. Also noteworthy is the lack of cyclicity in DC animals which was corrected after exposure to male odors.

Olfactory cortex acts on the hypothalamus via the pyriform cortex (4) while the visual may be seen to act via the retinohypothalamic pathways (17). In the DC animals, all cortical tissues have been removed including the hippocampus. The effect on the amygdala which is strictly speaking not part of the cortex, cannot presently be determined. However, it is well known that the hippocampus is part of the primitive cortex- archicortex which in the rat probably control more body areas than the neocortex. The effect of its ablation will be the lack of control of progesterone positive feed back via LH. Stimulation of the hypothalamus via the hippocampus is therefore totally removed. The net effect will be the abolition of the estros cycle.

We can only conjecture at the moment on the hormone profile in DC rats adjudged from the vaginal smears. It is probable that those rats with estros like smears have very low levels of circulating estrogen which is normally associated with such hormone profile (18). Those with diestrus-proestrus pattern would then probably have high levels of circulating estrogen (18). Modern studies have shown that the rat produces sex steroids (estrogen and progesterone) locally in the reproductive tract (19). Ogunranti (19) suggested that these may influence central control of reproductive function via LH feedback. It is difficult at the moment to determine the role of these local steroids (and peptides) in the decorticate rat but these could be useful studies in the future.

CONCLUSION

The famale rat is quite capable of existence without a functioning cortex, but its reproductive spontaneous cyclicity is abolished probably due mainly to low olfactory stimuli which may be followed by visual and then other exteroceptive factors. It is either capable of fixing its cyclicity on anestrous pattern or a diestrous pattern may be influenced by abnormal hormone steroid profile since there is tendency for corpus luteum maintenance in DC rats with damaged olfactory cortical apparatus. The DC rat however is quite capable of becoming pregnant but must contend with problem of nursing its young.

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