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Basal plasma corticosterone level after bilateral selective lesions of the olfactory pathways in the rat

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Summary. In comparison to control rats, basal plasma corticosterone level and lactacidemia significantly increased in rats submitted to a bilateral lesion of the lateral olfactory tract and/or the anterior branch of the anterior commissure. Only the lesion of the anterior branch of the anterior commissure induced hyperglycemia; that of the lateral olfactory tract exerted an opposite effect.
Key words. Rat; olfactory pathway selective lesions; plasma corticosterone; lactacidemia; glycemia; adrenal glands.

Olfactory deutoneurons (mitral and tufted cells) located in the olfactory bulb project into the higher nervous centers either directly via the lateral olfactory tract or indirectly via the anterior branch of the anterior commissure and the medial forebrain bundle[1]. Olfactory bulbectomy disturbs emotional reactivity[2,3] and induces either an increase in plasma corticosterone level[4] and adrenal gland weight[5] or the reverse[6]; such results might be due to variations in the extent of lesions, in age, or in the sex (male[4,5], female[6]) of the rats used. The characteristic emotional reactions elicited in sham-operated rats by biologically meaningful odorants (predator or congener odors) are no longer observed after a bilateral section of the lateral olfactory tract, alone[7], or associated with a bilateral lesion of the anterior branch of the anterior commissure[8].

In the latter case, only the medial forebrain bundle remains functional; we have never lesioned it as it is anatomically more diffuse and is involved in various kinds of behavior. On the contrary, after bilateral lesion of the anterior branch of the anterior commissure alone, the reactivity to the odorants slightly increases[8]. Moreover, lateral olfactory tract sections delayed an operant conditioning learning[9] (thirsty rats had to enter and go through a runway for a water reward in less than 6 s). Rats with a lesioned anterior commissure show a decreased ability to exhibit this well-learned behavior again after having been disturbed by an odorous stimulation once during the test (3 or 4 days of conditioning were necessary instead of 1 day at most for sham-operated rats)[9]. Such behavioral alterations are similar to those induced by olfactory bulbectomy[10]. However, the selective influence of each olfactory pathway on the corticoadrenal function remains unknown. The experiment reported here was carried out in order to determine which pathways are responsible for the olfactory bulbectomy effect on corticosterone secretion and on adrenal gland weights.

Materials and methods. Forty male Wistar SPF rats were used. On arrival in the laboratory they were housed in individual cages in the same room and exposed to the natural light-dark cycle in order to obtain experimental conditions identical to those of the behavioral tests[7-9]. Food and water were given ad libitum. The selective olfactory pathway lesions were performed under sodium pentobarbital anesthesia (37.5 mg/kg i.p.) according to the techniques previously described[7,8].

Four groups of 10 rats each were prepared. The control group underwent a sham-operation, i.e. the same procedure (anesthesia, surgery and thus operating stress) as the lesioned animals without lesioning the two olfactory pathways. The bLOT group underwent a bilateral section of the lateral olfactory tract, the bAC group, a bilateral lesion of the anterior branch of the anterior commissure and the bCL group a bilateral section of the lateral olfactory tract, followed two weeks later by a bilateral lesion of the anterior branch of the anterior commissure. Lateral olfactory tract sections were performed on 6-week-old rats, lesions of the anterior branch of the anterior commissure and sham-operations on 8-week-old rats. Then the animals were allowed to recover from surgery during 3 or 4 weeks in order to achieve experimental conditions similar to those of the behavioral tests[7-9]. No anatomical or behavioral recovery occurred during these tests.

When they were 10–11 weeks old the rats were decapitated after 30 s of ether anesthesia, which might slightly increase blood glucose, as reported in the dog[11]. Blood was always collected between 08.00 and 10.00 when the plasma corticosterone level is lowest[12], in order to detect more easily an increase in the corticosterone level induced by the olfactory lesions. Blood samples were taken in order to determine glucose[13] and lactic acid[14] (variations of these parameters being known to be correlated with metabolic disequilibrium, induced in particular by dysfunction of hypothalamo-pituitary-adrenal axis hormones). Plasma

Table 1. Plasma corticosterone, glycemia and lactacidemia in the four groups of rats studied. (Statistical details are given in the text)

Groups	Number of rats	Plasma corticosterone (nmol/l plasma)	Glycemia (mmol/l blood)	Lactacidemia (mmol/l blood)
Control	10	70 ± 31	6.08 ± 0.28	2.52 ± 0.67
bLOT	10	465 ± 112	5.35 ± 0.14	9.74 ± 1.53
bAC	10	1940 ± 148	7.83 ± 0.37	14.91 ± 0.96
bCL	10	500 ± 143	6.62 ± 0.34	6.07 ± 0.99

Table 2. Body and adrenal weights, and ratio of the cortico-adrenal area to the adrenal gland area of the four groups of rats tested. (Statistical results are given in the text)

Groups	Number of rats	Body weight (g)	Adrenal gland weight (mg)	Relative adrenal gland weight (mg/100 g body)	Number of rats	Cortico-adrenal area/ adrenal gland area
Control	10	293 ± 5.8	70.06 ± 3.10	23.90 ± 0.93	5	0.774 ± 0.020
bLOT	10	282 ± 9.5	65.05 ± 3.95	23.06 ± 1.15	5	0.834 ± 0.012
bAC	10	295 ± 5.1	76.35 ± 3.03	25.96 ± 1.12	5	0.861 ± 0.020
bCL	10	274 ± 7.1	76.28 ± 3.76	27.96 ± 1.49	5	0.821 ± 0.021

was stored at −30 °C. Plasma corticosterone levels were simultaneously determined for all rats by a modified[16] radioimmunoassay method[15]. The cortisol rabbit antibodies solution was furnished by New England Nuclear (RIANEN cortisol R.I.A.). The ^{3}H-labeled hormone was purchased from N.E.N. Analyses were made with the antibody at a dilution giving 50% binding of the ligand in the absence of unlabeled steroid. Cortisol exhibited 50% displacement and progesterone 2%. The standards used contained 0–50 µg/100 ml of corticosterone. Interassay and intraassay coefficients of variation given for this method are about 6.0% and 3.8% respectively (our results give an intraassay coefficient of variation = 4%). The plasma samples were assayed twice. Adrenal glands were removed and weighed and their relative weights (mg/100 g b.wt) were calculated. They were then kept in a 10% formalin solution and cresyl-violet stained frozen serial sections (20 µm) were prepared from half of the animals used. Sections where both the adrenal gland cortex and medulla were observed simultaneously were used in order to determine the respective areas of the whole gland and of its cortical part (the glandular sections were considered as ellipses and their two axes measured with a binocular magnifying glass micrometer). About 30 sections were studied in each group. The 40 rat brains were removed and fixed in a 10% formalin solution. The olfactory pathway lesions were examined for completeness in cresyl-violet stained frozen serial sections (100 µm) as previously described[7,8]. The sham-operated rat brains were also examined in the same way. Results were compared by using first the analysis of variance and then the Newman-Keuls test (if significant differences were noted).

Results. As the olfactory pathway lesions were complete in all the lesioned rats, results from all the animals could be considered. Mean values ± SEM are summarized in tables 1 and 2.

Plasma corticosterone: A significant effect ($p < 0.001$) of the olfactory pathway lesions was found by analysis of variance of the plasma corticosterone levels. The levels were significantly enhanced in all the lesioned groups in comparison with the controls ($p < 0.01$). They were similar in the bLOT and bCL groups, but the largest increase was noted in the bAC group which was also significantly different from bLOT and bCL groups ($p < 0.01$).

Glycemia: Levels of the four groups were significantly different ($p < 0.001$). The Newman-Keuls test showed a significant difference between the control and bCL groups ($p < 0.05$). Glycemia was significantly decreased in the bLOT group and significantly increased in the bAC group in comparison with the two other groups ($p < 0.01$).

Lactacidemia: The lesions induced a significant increase in lactacidemia ($p < 0.001$). The pair comparisons showed that each group was different from all the others ($p < 0.01$). Lactacidemia increased after an olfactory pathway lesion and especially after a lesion of the anterior branch of the anterior commissure.

Body weights: The analysis of variance indicated a significant difference ($p < 0.001$) between the groups, and the Newman-Keuls test showed that the bAC and control groups had a significantly heavier body weight than the bCL ($p < 0.01$) and the bLOT rats ($p < 0.05$).

Relative adrenal gland weights (mg/100 g b. wt): The analysis of variance exhibited a significant difference ($p < 0.025$). The ratios calculated for the bCL and bAC groups were significantly greater than those of control and bLOT rats ($p < 0.05$). It is noticeable that this increase is not an artefact resulting from the lower body weight of the bCL rats since the mean absolute adrenal gland weight was also greater in the bCL and bAC groups than in the other two ($p < 0.01$).

Ratio of the cortico-adrenal area to the adrenal gland area: The ratio was larger in the lesioned groups with enhanced hormonal level as compared to the control rats. However, no statistical analysis of the data could be performed, since significant inter-individual variations were observed within the 3 groups.

Discussion. A possible effect of the ether anesthesia can be discounted since its duration was much shorter than that (> 2 min) required to induce an increase in the corticosterone level[17], and since it did not influence the control group. The plasma corticosterone level was enhanced in the three lesioned groups of rats, especially in the bAC group. This increase agrees with results of Cairncross et al.[4]: corticosterone levels determined in plasma of individually housed 3-month-old male rats, (stressed or not) are similar to our results (bLOT or bCL rats and bAC rats respectively). Thus, bulbectomy and lateral olfactory tract lesion exert an identical influence. After both these lesions, rats behave as if they were anosmic[10,7]. The loss of olfactory information emanating from the environment might act as a stressor and thus would slightly activate the hypothalamo-pituitary-adrenal axis. Even if bAC rats cannot be considered to be chronically stressed animals, greatly enhanced glycemia and lactacidemia indicate a profound disturbance of glucose metabolism[18,19]. This large increase in lactacidemia can also be correlated with rat hyperreactivity[8,9].

In the rat, the perceived stimulation quantity and the corticosterone level are positively correlated[20]. After a bilateral lesion of the anterior branch of the anterior commissure, the usual olfactory deutoneuron habituation to repetitive odorous stimulation is no longer observed[8]; thus numerous signals are permanently transmitted to the piriform cortex and higher nervous centers via the lateral olfactory tract[21]. In the monkey the cortisol level is increased by electrical stimulation of various limbic areas (in particular of the piriform cortex[22]). Thus, a lesion of the anterior branch of the anterior commissure might greatly enhance the olfactory information reaching the higher nervous centers, particularly the hypothalamus. Such a process cannot function in the bCL rats since olfactory inputs cannot reach the olfactory centers. bCL animals, like the bLOT rats, behaved as if they were anosmic[8], and their plasma corticosterone levels were similar. In bCL rats glycemia is slightly enhanced in comparison to the control group but less than in the bAC group. The lateral olfactory tract section alone increases the corticosterone level but decreases glycemia. Thus, it can reduce the stimulating effect of the lesion of the anterior branch of the anterior commissure on the hypothalamo-pituitary-adrenal axis and on carbohydrate metabolism. Thus, additional evidence is provided for a functional duality of these two olfactory pathways[7,8,23]. These results are also in agreement with the observations of Sieck et al.[3]. The extent of the olfactory lesion (lateral olfactory tract and anterior olfactory nucleus where the anterior commissure originates) and the behavioral and biochemical changes are positively correlated. The medial forebrain bundle, involved in the mediation of the cortico-adrenal responses[24], might only be the final common pathway by which the olfactory inputs reach the hypothalamus.

The complex relation between olfaction and the hypothalamo-pituitary-adrenal axis is underlined. Even if the three olfactory pathways seem to be involved, the anterior branch of the anterior commissure might play a preponderant role in an inhibitory olfactory control of the hypothalamo-pituitary-adrenal axis in the intact rat. However, the origin of the corticosterone level increases after an olfactory lesion remains unknown; it may depend upon several factors which have yet to be studied.

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Suppression of annual plasma testosterone and thyroxine cycles in the edible dormouse *Glis glis* under constant photoperiod at 24 °C

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Summary. Exposure of male edible dormice all year round to an unvarying photoperiod and warm temperature disrupted their biological cycles; hibernation was almost completely suppressed, and short lived infradian cycles of body weight, and of plasma testosterone and thyroxine were measured instead of the normal annual pattern.
Key words. Hibernation; annual cycles; reproduction; thyroid.

Hibernators have long served as experimental models for the exploration of thermoregulatory mechanisms[1]. In addition, several species of hibernating mammals have been found very suitable for studies of the mechanisms underlying annual biological cycles. Most of the species so far studied in this respect have been shown to maintain endogenous circannual cycles of hibernation and body weight under a constant photoperiod (12 h light and 12 h dark) and cold temperature. They include the European hedgehog *Erinaceus europaeus*[2], the woodchuck *Marmota monax*[3], the chipmunks *Eutamias* and *Tamias striatus*[4], the golden mantled squirrel *Citellus citellus*[5], and other ground squirrels[6], the European hamster *Cricetus cricetus*[7] and the meadow jumping mouse *Zapus hudsonius*[8]. Under similar conditions, European hedgehogs[9] and the garden dormouse *Eliomys quercinus*[10] also displayed circannual cycles for testosterone levels. On the other hand the edible dormouse *Glis glis* exhibited a different regulatory pattern since its response to a constant cold environment and an unvarying photoperiod was the disappearance of annual rhythmicity, but persisting infradian cycles comprising periods of a few weeks or months observed for hibernation and body weight[11-13] and for plasma testosterone and thyroxine[14].

Since edible dormice also displayed the special characteristic of requiring normothermia as a prerequisite for any increase in their plasma testosterone and thyroxine titers[15], the present experiment was designed to explore the annual pattern of both these hormonal secretions, when animals were kept in a warm instead of a cold environment.

Material and methods. Eleven adult male edible dormice were kept from November 1982 through November 1983 in a room equipped with an automatic device providing a photoperiod of 12 h light (100 lux) and 12 h darkness; the temperature was maintained at 24 ± 0.2 °C. Animals were housed singly in wire mesh cages (65 × 34 × 30 cm) containing a nest-box furnished with straw and an automatic device providing drinking water. They had free access to a standard hamster chow (Provimi, Paris) and were given a few apples twice a week. Individual wake/dormancy behavior was checked daily. In addition, on a fixed date and hour each month, animals were weighed and their rectal temperature quickly measured with a Digi-sense thermometer; 1 ml blood samples were collected by heart puncture, centrifuged and stored at −30 °C. Temperature measurement, heart puncture and blood sampling were performed under fluothane anesthesia and lasted less than 2 min. Plasma testosterone was measured with RIA kits from the Commissariat à l'Energie Atomique (Saclay, France), and thyroxine levels were assayed by the radio-competition method as in earlier experiments[14,15]. Control dormice were kept under natural temperature conditions and a 12 h light/12 h dark schedule. Results are expressed as means ±SEM. For statistical comparisons, both within this experimental group and with earlier experiments, we used Student's t-tests and one-way ANOVA followed by Fisher's or