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MODULATION OF MITOGEN-INDUCED LYMPHOPROLIFERATION BY CEREBRAL NEOCORTEX

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Summary

The influence of the cerebral neocortex on the immunological status was studied in groups of C_3H/He mice after lesioning the right or left fronto-parietal cortex. In left lesioned mice, mitogenesis induced either by phytohemagglutinin (PHA) or concanavalin A (Con A) was depressed by about 50% compared to controls. On the contrary, in animals with right lesions, T cell mitogenesis was enhanced by about 140% as compared to controls and by 220-300% as compared to that observed in left lesioned animals. Mitogenesis of B cells induced by lipopolysaccharide was modified by cortical lesions in exactly the same way as that of T cell proliferation although not reaching statistical significance. These results confirm the lateralization in the cortical modulation of the immune system.

The immune system has classically been considered as an autoregulated system. It is known at the present time to be connected with the central nervous system (CNS). Stimulations or lesions of different hypothalamic areas modify various aspects of the immune response (1,5). Relationships between the CNS and the immune system are mediated by hormones from the hypothalamo-pituitary axis (6) and by the sympathetic nervous system (7) which are known to have regulatory roles on the immune response, possibly through their activity at the level of lymphocyte receptors (8). Opioids which are active on several lymphocyte functions in vitro (9) could also represent an important link between the CNS and the immune system. Conversely, the immune system could modify the activity of the CNS possibly by means of lymphokines (10). Furthermore, lymphocytes have been demonstrated to produce ACTH and endorphin-like substances (11,13).

Recently, Renoux et al.(14) reported the influence of the cortex on T-lymphocyte responses. Lesions of the left fronto-parietal cortex in mice lead to depression of T lymphocyte functions such as T cell mitogenesis, responses to alloantigens and IgG antibody production. On the contrary, lesions of the right cortex are accompanied by an augmentation of the same T cell functions. The lesions of the cortex appear not to modify B cell and macrophage functions. These results not only confirm the existence of modulatory brain influence on immune functions, especially T cell functions, but also show that such influences are subjected to lateralization. Nevertheless, unilateral cortical lesions have been reported by Nance et

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al.(15) to have no detectable effects on mitogen induced T cell proliferation. It should therefore be important to confirm the possible lateralization of the immunomodulatory activities of the cortex.

In the experiments reported here, thymus weight, serum antibody levels, and T- and B-cell mitogenesis have been determined in C_3H mice in which the right or left fronto-parietal cortex was removed. No difference was observed in thymus weight and antibody levels between lesioned and control animals but T-as well as B-cell mitogen induced proliferation was increased in animals with right lesions and depressed in animals with left lesions as compared to sham-operated controls.

Material and Methods

Animals. Female C_3H/He mice, purchased from the "Centre d'Elevage Janvier"(Le Genest, France), were housed 5 per cage in a room climatically controlled and with light on from 8.00 a.m. to 8.00 p.m. They were fed laboratory chow and water ad libitum.

Treatment of animals. Surgical lesions were performed in mice anesthetized by intraperitoneal injection of thiopental (70 mg/kg). The scalp was reclined and the skull overlying the cerebral cortex was removed, care being taken not to damage the superior sagittal sinus. Portions of either the right or left cerebral cortex were removed by knife cuts. The lesions involved dorsal and lateral parts of the frontal, parietal and occipital cortex (Fig.1) without penetrating the corpus callosum or the hippocampus (Fig.2). Animals did not receive antibiotics after surgery. Control animals had only the scalp reclined. After a six week interval, the three groups of 10 animals were immunized i.p. with 10^8 sheep red blood cells. Four days later, they were bled for hemagglutination tests and killed. The thymus was removed for weight determination and the spleen was removed for mitogenesis assays.

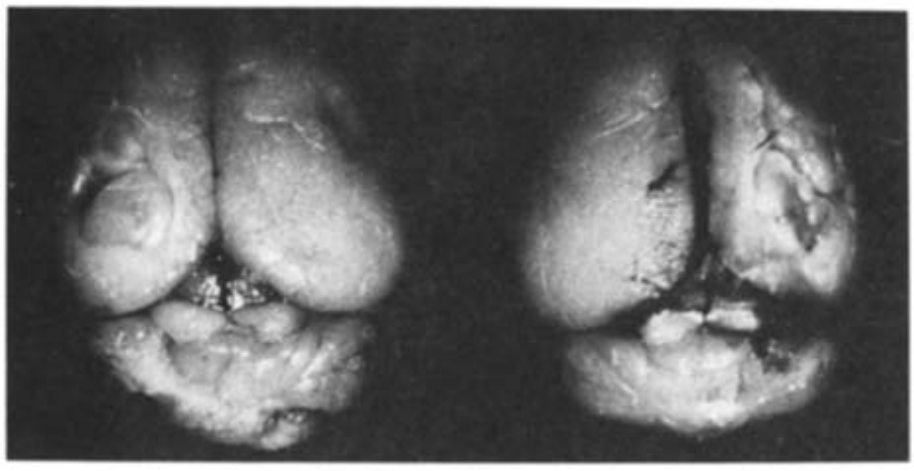

Fig.1 : Left- and right-lesioned brains

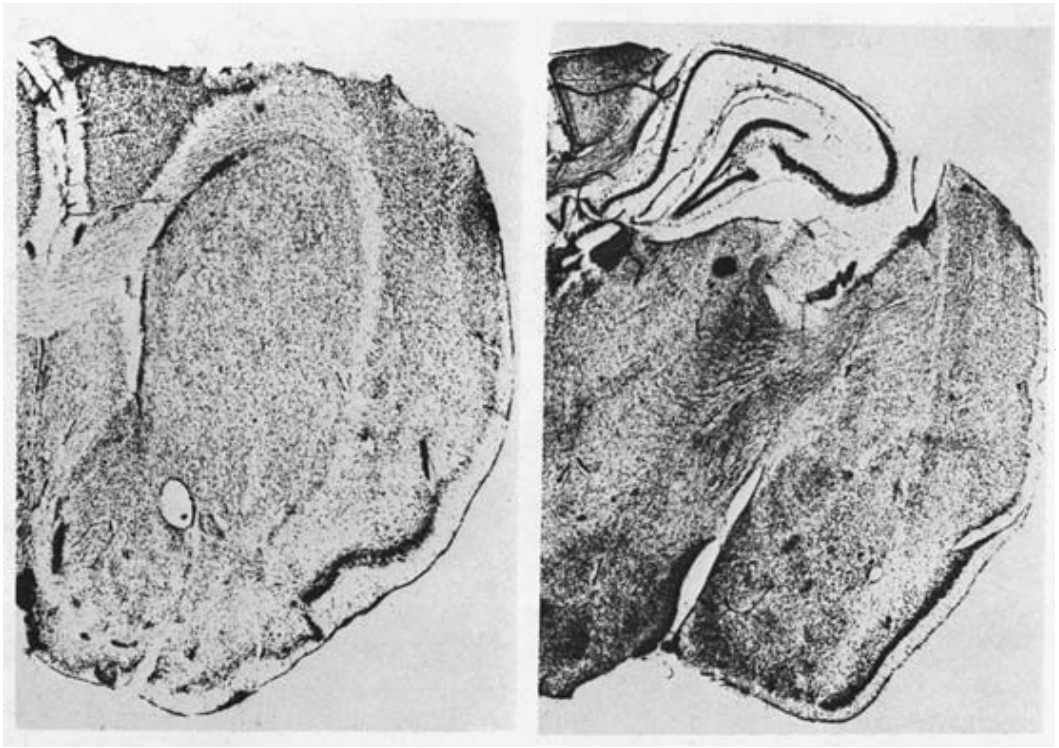

Fig.2 : Microscopic sections of a left-lesioned brain showing no lesion of the corpus callosum (left) or of the hippocampus (right)

Serum antibody level determination. Hemagglutination assays were performed in serums previously decomplemented at 56°C for 30 min according to standard procedures by doubling dilutions in microtiter trays.

Culture technique. RPMI-1640 (Gibco, Glasgow, UK) supplemented with 5% foetal calf serum (Gibco) and 1% antibiotic antimycotic solution (Gibco) was used throughout the experiments. Phytohemagglutinin (PHA-P) and Concanavalin A (Con A) were purchased from Difco (Detroit, Mi.,USA) and LPS of Escherichia coli from Sigma (St Louis, Mo.,USA). Spleen cells were dissociated in medium and after three washes adjusted to $2x10^6$ viable cells per ml. Cultures were performed in microtest II plates (Falcon, Cockeysville, Md., USA). Triplicates of spleen cell suspensions ($2x10^5$ cells per well) were cultured for four days in the presence of 50 ul of diluted mitogens in a humidified atmosphere of 5% CO_2. Concentrations of mitogens are expressed as final concentrations in the culture medium. Twenty-four hr before the termination of the culture 2 uCi of ^{3}H-thymidine (specific activity of 5 Ci $mMol^{-1}$, CEA, France) was added in a volume of 50 ul. Cells were collected on fiberglass strips using a multiple harvester (Mash 11, Microbiological Associates, Bethesda, Md.,USA). The filters were dried and counted in 5 ml of PPO-POPOP, in a liquid scintillation counter.

The results are expressed as stimulation indexes calculated for each animal as follows :

$$SI = \frac{\text{mean cpm stimulated culture}}{\text{mean cpm unstimulated culture}}$$

Results

Lesions of right or left neocortex did not significantly modify thymus weight (26 mg ± 8 and 29 mg ± 9, respectively) as compared to controls (22 mg±6). Antibody levels, as measured by hemagglutination tests, did not differ among the groups. Log_2 hemagglutination titers for right-operated (gr R), left-operated (gr L) or controls (gr C) were 5.8 ± 5.7, 5.6 ± 5.55 and 6.8 ± 6.6, respectively. In contrast, cortex lesions were correlated with modifications of mitogen-induced lymphoproliferation. Mitogenesis induced by 1 or 2 ug of Con A (Fig.3) was enhanced in mice of group R and depressed in animals of group L as compared to controls. Significant differences ($p<.05$ using standard ANOVA) were observed between groups R and L. Likewise, PHA-induced lymphoproliferation was enhanced in group R and depressed in group L as compared to controls (Fig.4). Differences between groups R and L were significant ($p<.05$ using the Kruskal-Wallis nonparametric test) when mitogenesis was induced with 16.7 ug/ml PHA, but not significant ($p = .08$) when using 8.3 ug/ml PHA. Similar results were observed when studyingg mitogenesis induced by LPS (Fig.5) but the differences were not significant ($p = .06$ and .14) when using 52 or 26 ug/ml LPS respectively.

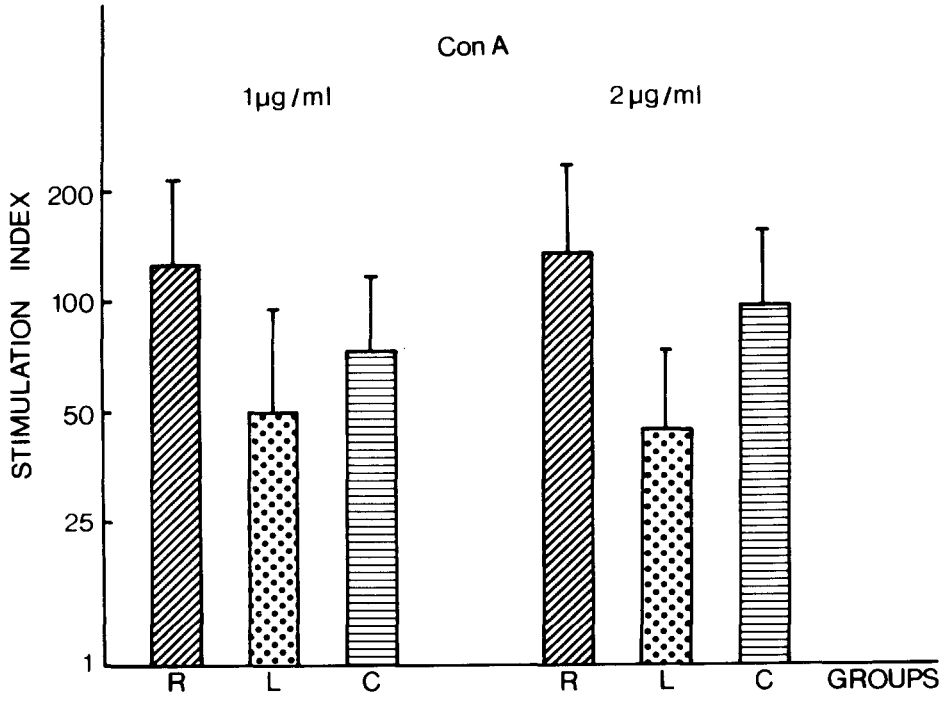

Fig.3 : Con A-induced spleen lymphoproliferation in groups of ten C_3H mice 6 weeks after lesioning the right (gr R) or the left (gr L) cortex. Gr C represents the sham-operated group.

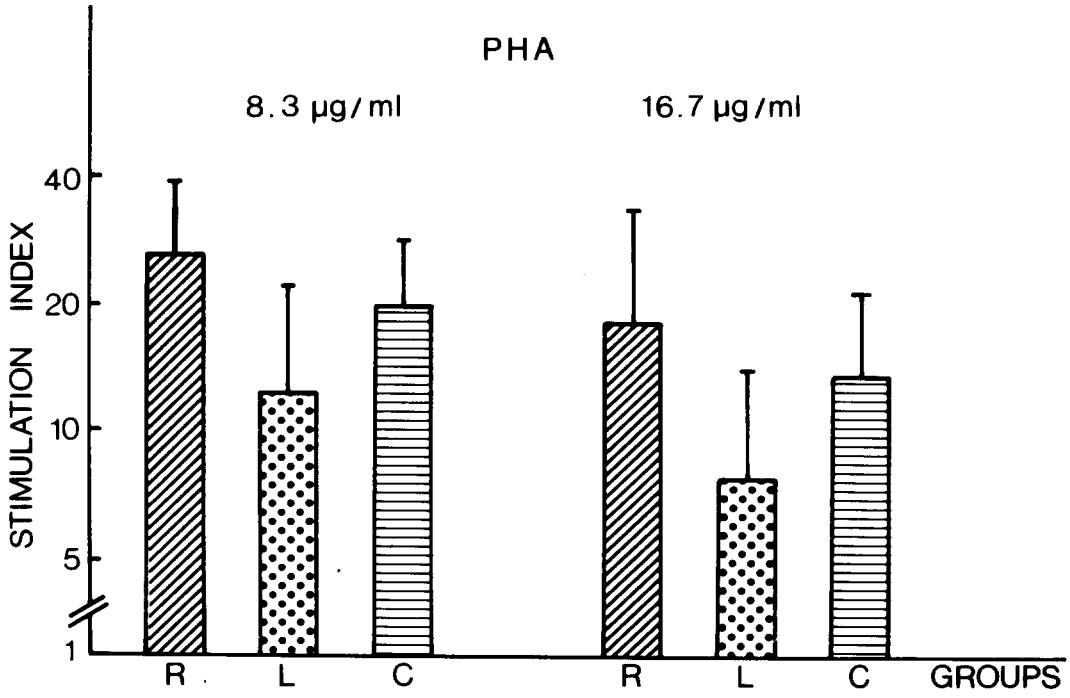

Fig.4 : PHA-induced spleen lymphoproliferation in groups of ten C_3H mice 6weeks afterlesioning the right (gr R) or the left (gr L) cortex. Gr C represents the sham-operated group.

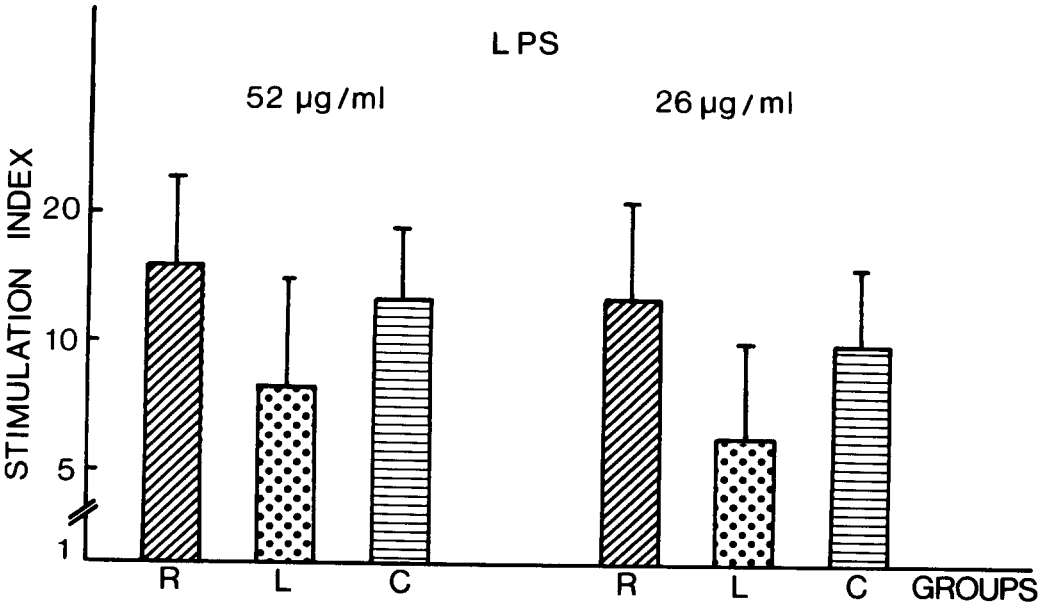

Fig.5 : LPS-induced spleen lymphoproliferation in groups of ten C_3H mice 6 weeks after lesioning the right (gr R) or the left (gr L) cortex. Gr C represents the sham-operated group.

Discussion

The influence of the cerebral neocortex on immunological status was studied in mice after lesioning the right or left fronto-parietal cortex. In the experiments reported here, no effect of cortical lesions on thymus weight could be observed. These results do not agree with those of Renoux et al.(14) who have reported a reduction or an augmentation of thymus weight in left-and right-lesioned mice, respectively. However, in the present experiments, there were important individual variations in thymus weight which may be responsible for our negative results. On the other hand, antibody levels were not modified after unilateral cortical lesions. These results are in agreement with those of Renoux and al.(14) who have shown that cortical lesions modify IgG antibody synthesis but do not affect IgM antibody production. Most of the hemagglutinating antibodies present 4 days after immunization belong to the IgM isotype which does not appeared to be altered by cortical lesions. By contrast, cortical lesions modified mitogen-induced lymphoproliferation of both T and B cells. In left-lesioned mice (gr L) mitogenesis of T cells induced either by PHA or Con A was depressed by about 50% as compared to controls (gr C). In animals with right cortical lesions (gr R), T cell proliferation assayed with PHA or Con A was enhanced by about 140% as compared to control and by 220-300% as compared to group L. These variations of mitogenesis were of the same extent as those described by Renoux et al.(14). Although not reaching statistical significance, mitogenesis of B cells induced by LPS was modified by cortical lesions exactly in the same way as that of T cell proliferation. Mice of group L showed B cell proliferation that was depressed by 60% as compared to controls (gr C). In animals of gr R, mitogenesis of B cells was enhanced by 120% as compared to controls and by about 200% as compared to group L. These results appear to be opposite to those of Renoux et al.(14) who reported that cortical lesions did not affect B cell mitogenesis induced by pokeweed mitogen instead of LPS.

Present results confirm those of Renoux et al.(14) in that the cerebral neocortex is involved in the control of the T cell lineage. To our knowledge, our report is the first confirmation of the hemispheric lateralization for the control of the immune system. Furthermore, our results point out the possible cortical modulation of B cell functions. Such a lateralization in the cortical regulation of the immune response could be responsible for the increased frequency of autoimmune diseases in left-handers (16). The mechanisms by which the cortex controls the immune system is not yet well known. The cortex has been reported to modify the synthesis or release of T-cell-inducing factors possibly through the activity of subcortical centers (17). Experiments to extend this phenomenon to other species and to study some of the mechanisms involved are now in progress.

Acknowledgment

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