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Short communication

Mental stimulation increases circulating CD4-positive T lymphocytes: a preliminary study

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Abstract

To stimulate the dorsolateral frontal cortex, 12 healthy, adult, human females played contract bridge for 1.5 h between initial and final blood sample collections. Flow cytometric analyses of samples, performed in triplicate, showed a significant increase in CD4-positive T lymphocytes. The dorsolateral frontal cortical thickness is significantly and bilaterally reduced in immune-incompetent female, nude mice. Thymic transplants reverse the deficient cortical thickness and CD4-positive cell numbers. © 2001 Published by Elsevier Science B.V.

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Many investigators have clearly demonstrated that manipulation of the highest cognitive region of the brain, the neocortex, has a role in immune regulation. Particular alteration of one system can subsequently affect the other. In the early 1980s lesion studies of the cerebral cortex resulted in enhancing or inhibiting effects on the immune system [1,14]. Ablation of the left dorsal and lateral frontal, parietal and occipital cortical areas in rodents caused decreased activities in T cells and natural killer cells. Lesions in the contralateral cortical areas increased the same immune cells.

Inspired by these initial investigations, we studied the congenitally athymic nude mouse to identify areas of the cerebral cortex that may be affected by the T cell-deficient state. Nude mice have few or no CD4-positive T lymphocytes.

In 1986, we published [9] our first research project dealing with the cerebral cortex and the immune system. Essentially this project demonstrated that the dorsolateral frontal cortex was bilaterally deficient, as measured by microscopic thickness [10], in the female, immune incompetent, nude mouse when compared with the cortical thickness of an immune competent mouse from the BALB/c strain. In 1996 and 1997, two more studies [11,12] confirmed this cortical deficiency and in addition demonstrated that cortical and blood immune deficiencies in the nude mouse could be reversed with thymic transplants.

All of these studies were performed on female nude or BALB/c mice. Results obtained from female C3H mice have been partially replicated in male mice for NK cell activity [6] and for lymphocyte proliferation in female Sprague–Dawley and male Wistar rats [2,13]. These findings with rodents suggest that cortical immune responses can be generalized across both sexes and in different species and possibly in human beings.

The functions of the dorsolateral frontal cortex in humans include working memory, changing set, judgment, initiative, planning ahead, sequencing data, etc. Some investigators have utilized the xenon dynamic single photon emission-computed tomography (SPECT) during the performance of the Wisconsin Card Sorting Test (WCST) to demonstrate activation of the dorsolateral

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frontal cortex in humans [16]. The WCST is a problem-solving, abstract reasoning test used as a sensitive indicator of frontal lobe integrity in humans. Individuals with dorsolateral prefrontal lesions do poorly on the WCST.

Berman and Weinberger [4] measured regional cerebral blood flow using xenon inhalation methods while normal subjects performed 10 different tasks including the WCST. The dorsolateral prefrontal cortex was activated by these tasks. Thus, the findings of several laboratories [3–5,7,16] have shown that regional activation of the frontal lobes can occur in response to cognitive challenges produced through performance of a standard neuropsychological card test.

In search of a well-known card playing experience, instead of the professionally known WCST, we chose the card game of contract bridge This game has the potential to stimulate many of the functions attributed to the dorsolateral frontal cortex, though it is not an established scientific way to activate the prefrontal cortex. We hypothesized that while individuals played bridge, this area of the cortex might be stimulated and possibly influence the production of T lymphocytes. Therefore, we planned to take blood samples before and after adult women played 1.5 h of contract bridge and to quantify the number of T lymphocytes in the blood samples, including CD3, CD4, CD8 and CD56 cell types.

The following procedures were designed to test our initial hypothesis. Twelve, adult, human, female subjects (age range 70–80 years) were easily recruited from the Orinda, California bridge clubs. (Our university Bridge Club had no female students, only males.) Females were preferred for two reasons: (1) because female mice were used in the initial studies by the French scientists studying cortical/immune interactions [14,15]; and (2) because all of our animal studies correlating the cerebral cortex with the immune system have been carried out on females [9,11,12]. The results of our prescreening questionnaire indicated that all the human, female subjects were free of neurological or systemic illnesses with implications for cerebral malfunction.

The ladies were divided into three groups of four women in each group for card playing. Ten milliliters of heparinized blood were drawn through antiseptic skin from the median cubital vein in each woman prior to playing contract bridge for 1.5 h; after this time another sample of 10 ml was collected. Within 2 h of collection, flow cytometer analysis of samples was performed in triplicate on an EPICS XL_MCL (Beckman-Coulter). Samples were analyzed using an argon-ion laser with fluorescence excitation at 488 nm. Fluorescence emission was collected at 525, 575, 610, and 675 nm, respectively.

At least 10 000 cells were collected per sample. The mean of the triplicate values for each parameter was subjected to both parametric and non-parametric measures for data analysis. Paired t-test analysis was performed on each of the pairs of samples to determine differences in cells positive for the four lymphocyte markers. In addition, a Wilcoxon signed ranks test and a sign test were used to determine increases of the cells in the post-sample compared to the pre-sample. A significance level of $P<0.05$ was used for the analyses.

Table 1
Mean values for percentage of antigen-bearing lymphocytes in paired samples

Antigen	n	Mean pre-sample	Mean post-sample	Mean difference	S.D.
CD3	12	68.43	68.85	0.43	3.30
CD 8	12	27.03	26.98	−0.04	3.37
CD 56	12	14.12	14.06	−0.06	3.81
CD 4	12	49.06	50.90	1.84*	3.53
Day 1	4	47.47	47.76	0.29	5.93
Day 2	4	52.07	53.92	1.85**	0.87
Day 3	4	47.65	51.03	3.38**	1.84

*Wilcoxon signed ranked test and sign test $P<0.05$; paired t-test $P<0.10$; **paired t-test $P<0.05$.

Table 1 offers the mean values for percentage of antigen bearing lymphocytes in paired samples. Fig. 1 presents the mean difference between paired samples of lymphocyte antigens. By comparing venous blood samples before and after 1.5 h of bridge playing, a significant increase was found in CD4-positive T lymphocytes as measured by both the Wilcoxon signed rank test ($Z=-2.040$; $P<0.041$) and the sign test ($P<0.039$) (see Fig. 1). Although there was only a trend indicating an increase in CD4 antigens as measured by the Paired t-test ($t(11)=1.809$; $P<0.098$), there was a significant interaction between day of the study and CD4 increase, with statistically significant increases occurring for subjects who participated on the last 2 days of the study (Day 2: $t(3)=4.272$; $P<0.024$; Day 3: $t(3)=3.686$; $P<0.035$). Only one subject was aberrant for day 1, preventing a significant finding for this group. No significant increases in CD3, CD8 or CD56 were detected as measured by the Wilcoxon signed rank, sign and paired t-tests (see Table 1). In addition, no significant CD4 cell

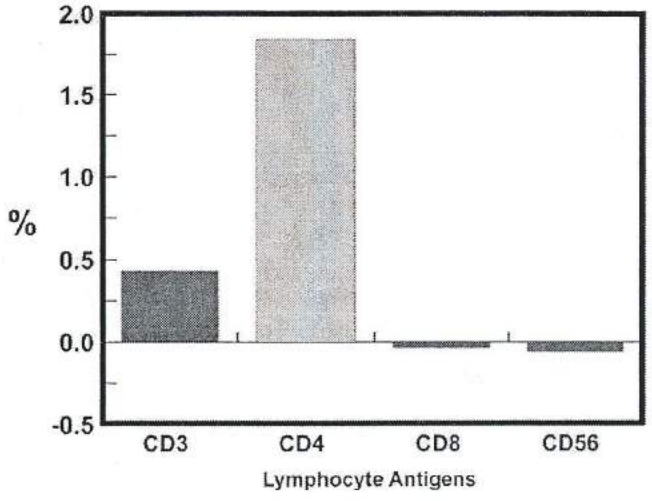

Fig. 1. Mean difference between paired samples.

increases were found between the two samples of the blood drawn from each of three women who did not play bridge, but only sat quietly listening to gentle music at the same time period during which the experimental group played cards. The lack of change in CD4 cell number with this group suggested that stress, due to the procedure for withdrawing of blood, was not a main factor.

This present *preliminary* experiment has indicated that numbers of human immune T cells, specifically CD4-positive T lymphocytes, can be stimulated by someone playing the card game of contract bridge. There is no doubt that serious bridge playing requires higher cognitive processes associated with the frontal areas of the cerebral cortex. Other mentally challenging activities could also bring about changes in the T lymphocyte counts. Further tests are needed. Our data suggest that people might be able to improve their immune functions with more purposeful demanding activities related to frontal lobe tasks.

The correlation between the psyche and the well-being of the individual has been recognized since the time of Hippocrates, but the possible interaction between regions of the cerebral cortex and the immune system was only brought to the attention of more recent investigators through the studies of Renoux et al. [14], Bardos et al. [1] and Renoux [15] in the early 1980s. They studied the effects of lesions in the right or left neocortex on the activity of natural killer cells and antibody-dependent, cell-mediated cytotoxicity of splenic T cells.

Many differences in the response of the immune system to these ablation studies have been presented and may be accounted for by varying factors including extent of the lesion and period of immune analyses following the lesions. In 1999, Davidson et al. [7] demonstrated with electrophysiological measures that activation of the prefrontal cortex influenced natural killer cell activity. These investigations clearly have demonstrated that manipulation of a higher cognitive region, the neocortex and more specifically the frontal cortex, has a definite role in immune regulation.

It is well known that busy, engaged people do not become ill as frequently as idle, inactive people. Positive thinking is reportedly beneficial to one's well being. Even prayer has been implied to have a positive effect on one's health. Are all of these statements true because an enhanced frontal lobe protects the individual through excitation of certain immune functions?

Uncited reference

[8]

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