

## Memorandum

Date September 9, 1982

From Deputy Director, Division of Electronic Products/BRH

Subject Bioeffects of Air Ions *A. Rechen*

To Director, Division of Electronic Products/BRH

I telephoned Michael Bissell, Ph.D., (Bldg. 36, Room 3D-04, NIMH, tel. 496-4361) on 9/8/82, to obtain a copy of his 1980 paper in Science, which claimed that air ion levels affect serotonin levels in rats.

Dr. Bissell is sending us two papers:

(1) "Environmental Influences on Serotonin and Cyclic Nucleotides in Rat Cerebral Cortex," by M.C. Diamond; J.R. Connor, Jr.; E.K. Orenberg; M. Bissell; M. Yost; A. Krueger. Science, Vol. 210, 7 November 1980, pp. 652-654.

This report was done while Dr. Bissell was at the Department of Dermatology, Stanford University School of Medicine, Stanford, CA. The other authors were at either the U of C, Berkeley, or the VA Medical Center, Martinez, CA (U of C, Davis).

Dr. Bissell remarked that he has since had second thoughts about the paper, and can think of a number of valid reasons other than air ions to account for the observed changes in serotonin levels. Serotonin is a general stress indicator, and extreme meticulous care is necessary in designing and conducting a controlled study.

(2) A draft of a consensus panel report he helped prepare, as a panel member, for the State of Minnesota. The report discusses the possible effects of air ions generated by power lines. Among other effects, near by farmers are claiming excessive abortion and malformation rates in their livestock. The Minnesota area, Dr. Bissell says, has a data base of detailed records of such occurrences, both before and after power line installation.

The panel report is being distributed in a few days to a worldwide group for peer review; when the reviewers comments are incorporated, the full report will be available.

Dr. Bissell also stated that he plans to attend the "Conference on Environmental Ions and Related Biological Effects," sponsored by the American Institute of Medical Climatology, 1023 Welsh Rd., Philadelphia, Pa., 19115. The Conference will be held on October 30, 1982, 9 a.m. - 4:45 p.m., Stratton Hall, Room 113, Drexel University, 32nd and Chestnut Sts., Philadelphia, Pa. 19104. Registration for non-members is \$35.00.

He was pleased to hear that we are planning to send at least one observer (Dr. Fred Prevo, DEP), and expressed a willingness to consult with us in the future.

Attachment

## Environmental Influences on Serotonin and Cyclic Nucleotides in Rat Cerebral Cortex

**Abstract.** *The response to different environmental conditions and negative air ions was investigated on cerebral cortical serotonin and cyclic nucleotides. The results indicated that negative air ions alter the weight of the cerebral cortex and that concentrations of serotonin and cyclic nucleotides can be altered both by different environments and by negative air ions. The data stress the importance of the role of the environment when studying the structure and chemistry of the cerebral cortex.*

The structure and chemistry of the cerebral cortex change constantly in response to modifications in the external and internal environment. Sensory stimuli from the external environment can either increase or decrease the size and chemical responses of the cerebral cortex, depending on the quantity and quality of the stimulus (1). Cortical morphology and chemistry can be altered by fluctuations in the internal environment brought about by such substances as hormones (2). The possibility that physical environmental stimuli other than those classically regarded as "sensory" (for example, electromagnetic waves) may also have such effects on the brain is beginning to receive some attention (3).

That atmospheric ionization may represent one such stimulus is suggested by the work of Sulman *et al.* (4), who studied the responses of groups of weather-sensitive individuals to changes in the ionization of air and noted, among other things, desynchronization and frequency shifts in the patterns of their electroencephalograms (5). We became interested in studying air ions because there are considerable shifts in the concentration

of air ions in the ambient atmosphere of our biosphere, resulting from the winds of ill repute, such as the foehn, chinook, and Santa Ana. The physical properties of small air ions are discussed by Krueger and Reed (6).

The concept that the known physiological and biochemical effects of air ions may depend on their ability to alter the metabolism of biogenic amines was introduced by Krueger and Smith (7). Subsequently, it has been shown that concentrations of serotonin in blood and brain respond to prevailing levels of atmospheric ions (8). There is evidence that the cyclic nucleotides participate in some of the metabolic events underlying synaptic transmission within the mammalian central nervous system and that changes in brain content of adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) may reflect interactions between some neurotransmitters and their synaptic receptors (9). Therefore, experiments were undertaken to determine whether the effect of negative ions on serotonin, on the putative second messenger cyclic AMP, and on cyclic GMP in the cerebral cortex depends on whether the animals

live in enriched or impoverished environments.

Groups of male Long-Evans rats were housed in enriched or impoverished environments, with and without increased levels of negative air ions. Nine litter-mate pairs of 6-day-old pups were separated and distributed among six mothers into two experimental environments: a multifamily enriched condition (MFEC) and a unifamily impoverished condition (UFIC) (10). In the MFEC, three mothers, each with three pups, were housed in a large cage (70 by 70 by 46 cm) filled with an assortment of objects with which to interact, referred to as "toys." In the UFIC, one mother was housed with her three pups in a small cage (32 by 28 by 20 cm) with no toys; there were three UFIC cages. The entire experiment with the increased negative ion atmosphere was replicated (there were 18 pups in the elevated ion group for the initial experiment and 18 for the replication experiment). Animals living in atmospheric conditions, that is, receiving air delivered by the building ventilation system and containing fewer than 100 positive ions per cubic centimeter of air, were grouped in a similar fashion in wire mesh enrichment cages and standard laboratory cages (18 pups for the atmospheric condition group).

All animals were disturbed for a few minutes each day while the toys were being changed in the enrichment cage and the sawdust trays were removed for cleaning in the small cages. The sawdust in the large cage was changed only three times each week. Animals were maintained on a 12-hour light-dark cycle and had free access to food and water. All pups lived in their respective housing from 6 to 26 days of age.

Both the MFEC and UFIC animals exposed to negative air ions lived in Lucite cages, with Lucite toys in the enrichment cage (Fig. 1). A grounded wire mesh floor was suspended over the sawdust waste collection tray at the cage bottom. A fan supplied equal amounts of air to each cage, and a filter was used to free the air from particulates.

An air ion density of  $1 \times 10^5$  small negative ions per cubic centimeter was maintained in both the large and small cages. Negative air ions were generated by corona discharge from Amcor Modulon power supplies and regulated in each cage by adjusting the ionization potential with separate variable transformers on the a-c lines of each generator. The ion density of the air in each cage was measured with the aid of a Royco volumetric counter and was correlated with the flow of current from the wire mesh floor to a

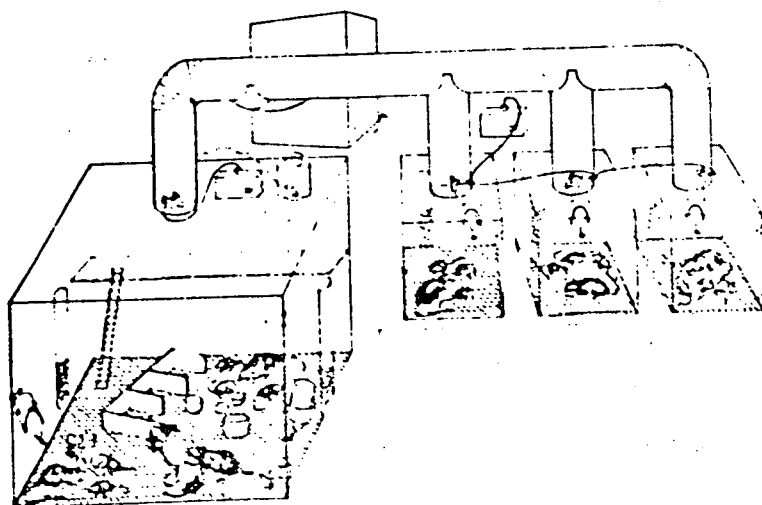


Fig. 1. The multifamily enriched condition with toys is shown in the left large cage and the unifamily impoverished condition is shown in each of the three small cages. The ion sources, transformers, and conduits for delivering the air to each cage are above each cage.

earth ground. Daily checks of the current flow to ground were made to ensure proper operation of the ionization equipment.

Before brain samples were dissected for chemical analysis, all animals were coded to prevent experimental bias. Uniform samples of somatosensory and occipital cortices were surgically removed from both hemispheres with the aid of a plastic calibrated T square (11). The samples were weighed on aluminum foil on a Mettler balance and immediately wrapped in foil and frozen on a block of dry ice. All procedures were accomplished within 4 minutes after the animals were killed by decapitation. All groups of animals were analyzed simultaneously.

Assay procedures for isolation, purification, and quantification of serotonin were as described in (12) and adapted for use for small brain regions (13). Determination of cyclic AMP was by radioimmunoassay as described in (14). The DNA was quantitated by fluorometric methods (15). Although brain wet weights varied, the total amount of DNA in the brain areas for the MFEC and UFIC animals was the same regardless of environmental conditions. Chemistry values are therefore reported per unit of DNA (16). Differences in biochemical values in brain regions of animals living in dif-

Table 1. Differences in wet weight of the cortex between rats receiving negative ions and those in atmospheric conditions. N.S., not significant.

Cortical area	Wet weight of cortex (mg)		Percent difference	P <sup>a</sup>
	Negative ions	Atmospheric conditions		
<i>Multi-family enriched condition</i>				
Somatosensory	61.75 ± 0.89 (N = 16) <sup>b</sup>	55.4 ± 1.04 (N = 8)	12	.05
Occipital	82.3 ± 1.5 (N = 17)	73.2 ± 1.54 (N = 8)	17	.001
<i>Unifamily impoverished condition</i>				
Somatosensory	58.65 ± 0.86 (N = 15)	56.4 ± 1.22 (N = 9)	4	N.S.
Occipital	78.8 ± 1.6 (N = 15)	73.6 ± 1.14 (N = 9)	7	.05

<sup>a</sup>Student's *t*-test, two-tailed. <sup>b</sup>Values of N may differ from original values owing to loss of animal or tissue during experimental procedures.

ferent environments with and without negative air ions were tested by analysis of covariance. Specific differences between mean biochemical levels were analyzed by Student's *t*-test, two-tailed.

The wet weights of both the somatosensory and occipital cortices were greater in rats receiving negative ions than in those living in atmospheric conditions (Table 1). The MFEC rats living in a negative ion atmosphere had significantly less serotonin (61 percent,  $P < .01$ ) and cyclic AMP (45 percent,  $P < .05$ ) in the somatosensory cortex than MFEC rats living in atmospheric conditions (Fig. 2). Serotonin and cyclic AMP concentrations in the occipital cortex were also significantly less (45 percent,  $P < .05$  and 35 percent,  $P < .05$ , respectively) in the MFEC rats receiving negative ions than in the MFEC rats living in atmospheric conditions (Fig. 3). It appears that, in the MFEC, negative ions prevent the increases in serotonin and cyclic AMP concentrations that occur in atmospheric conditions. The cyclic GMP levels did increase, though not significantly, in the somatosensory cortex and in the occipital cortex of both the MFEC and UFIC rats receiving negative ions in comparison with rats living in atmospheric conditions (Figs. 2 and 3).

Our data on serotonin are consistent with other reports that negative air ions decrease brain serotonin. Gilbert (17) used negative ions to reduce emotionality caused by isolation; the reduction in emotionality paralleled a decrease in serotonin. Oliverau (18) found that brief exposure of rats to  $1.5 \times 10^5$  small negative ions per cubic centimeter of air produced an anxiolytic effect in a stressful situation. He considered the effect to be in agreement with the serotonin hypothesis on the action of air ions.

Since putative biogenic amine transmitters can activate adenylate cyclase, it is possible that the changes in cyclic AMP content of the brain that we observed may be a consequence of a rela-

tive decrease in the release of serotonin. The changes might also result from an altered sensitivity or responsiveness of the synthetic enzyme adenylate cyclase or altered activity of phosphodiesterase. In most instances, the pattern of change in the content of cyclic AMP was in the same direction as the change in serotonin. If changes in cyclic AMP are serotonin-dependent, cyclic AMP might reflect the metabolic alteration in serotonin effected by negative air ions, as well as the living group situation.

The content of cyclic GMP was relatively unchanged by atmospheric and environmental state. This may reflect regional variations in the significance of cyclic GMP and also indicate the neuronal cells selectively control steady-state tissue levels of the two cyclic nucle-

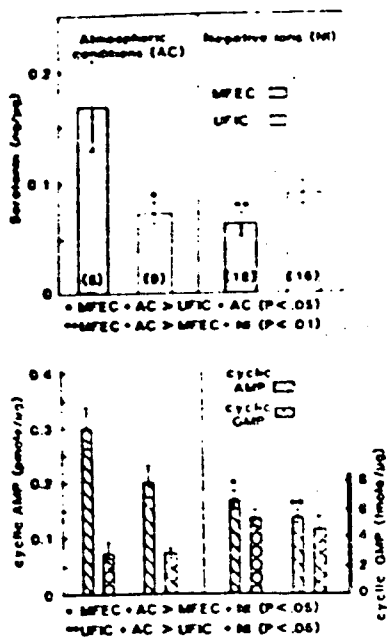


Fig. 2. Amounts of serotonin, cyclic AMP, and cyclic GMP (per microgram of DNA) in the somatosensory cortex of animals in enriched and impoverished environments with atmospheric conditions or with negative air ions.

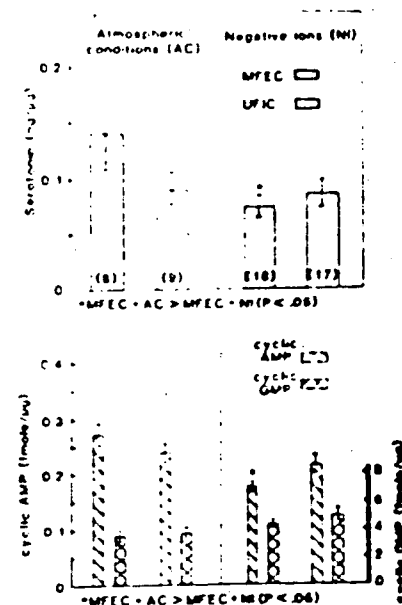


Fig. 3. Amounts of serotonin, cyclic AMP, and cyclic GMP (per microgram of DNA) in the occipital cortex of animals in enriched and impoverished environments with atmospheric conditions or with negative air ions.

otides by independent regulatory mechanisms (19).

Six-day-old rats were chosen as experimental animals in this study to include potential effects of air ions on neural development. There appears to be a serotonin-dependent adenylate cyclase system in very young animals, which decreases in sensitivity with age (20). Our results with serotonin and cyclic AMP may be due to this coupling. It is conceivable, however, that negative air ions could have shifted the apparent developmental stage of the rats in this study by a primary effect on the concentration of some hormone, such as prolactin, sex hormone, or serotonin-derived melatonin. A direct effect of small air ions on prolactin levels was proposed by Oliverau in his study of the effects of air ions on the spontaneous movements of amphibian larvae (21). A direct hormonal effect of this kind in our system may have caused the increased cortical weights and decreased cortical levels of a second-messenger cyclic AMP reported here. Comparison of effects of negative and positive ions and different environments on various transmitters should show how the responses are related to the developmental stage of the animal.

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#### References and Notes

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16. The mean wet weight ( $\pm$  standard error) for

brain samples for the somatosensory region was  $58.8 \pm 0.6$  mg ( $N = 51$ ) and for the occipital region  $77.5 \pm 0.9$  mg ( $N = 50$ ). The average total DNA content for somatosensory samples was  $208.4 \pm 12.0$   $\mu$ g of DNA and for the occipital samples,  $272.3 \pm 11.0$   $\mu$ g of DNA. Therefore there was an average 3.5  $\mu$ g of DNA per milligram wet weight for both brain regions.

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22. We are greatly indebted to Marie Hebert for her skilful dissection of brain tissue and to Rosalie Greer and Chong L. Lee for their assistance.

27 March 1980, revised 14 May 1980

## Nigral Dopamine Neurons: Intracellular Recording and Identification with L-Dopa Injection and Histochemistry

**Abstract.** Intracellular recordings *in vivo* were obtained from dopamine-containing neurons of the rat substantia nigra. These neurons were identified electrophysiologically by antidromic activation and histochemically by L-dopa injection and subsequent fluorescence histochemistry. Extracellular spikes and antidromic conduction velocity of the neurons were identical to those previously described for putative dopaminergic neurons. Spontaneous intracellular fast potentials, slow depolarizations during burst firing, and spike prepotentials were observed.

The nigrostriatal dopaminergic system has been studied intensively because of its participation in the pathogenesis of Parkinson's disease (1) and its role in mediating the neurological side effects of antipsychotic drugs (2). Mapping of this system by fluorescence histochemical techniques has identified the location of these cells and made it possible to record extracellularly *in vivo* from the area in which these cells are contained. Dopamine-containing neurons of the substantia nigra exist in a thin band on its dorsal edge (3) and are intermixed with neurons that do not contain dopamine (4, 5). Hence, to date, electrophysiological identification of these cells has depended on indirect criteria (6). Extracellular administration of L-dopa followed by processing of the brain slices for catecholamine fluorescence (7) was one of these criteria, because only the dopamine-containing neurons in this brain region contain aromatic amino acid decarboxylase (the enzyme necessary for conversion of L-dopa to dopamine) and only the dopamine reacts with formaldehyde vapor or glyoxylic acid to form fluorescent compounds. Thus, the increased fluorescence of these neurons after L-dopa iontophoresis (resulting from its uptake and conversion to dopamine) demonstrated conclusively that the recording electrode was in the vicinity of these neurons. However, because the L-

dopa was administered extracellularly, it did not allow identification of the specific cell from which the recording was made.

Extracellular recordings of these indirectly identified dopamine-containing neurons have been useful in studying the modes of action of various pharmacological agents, such as the antipsychotic drugs and dopamine agonists (for example, *d*- and *l*-amphetamine and apomorphine), as well as in studying some aspects of their neurophysiological functioning (8, 9). However, the exact membrane mechanisms underlying dopaminergic cell functioning and the effects of drugs on them cannot be inferred from the results of these studies; only intracellular recording can demonstrate the precise synaptic and ionic mechanisms. We report here intracellular recordings from dopamine-containing neurons of the rat substantia nigra. The cells were positively identified as dopaminergic by intracellular iontophoresis of L-dopa at the end of the recording and subsequent processing of the brain for fluorescence histochemistry.

Rats were used as experimental subjects (10). Intracellular recordings (11) were made from presumed dopamine-containing neurons of the zona compacta region of the substantia nigra ( $N = 50$ ). The cells were tentatively identified before electrode penetration by their extracellular firing pattern, waveform, rate of