in stress effects on B cell functions must be further investigated. Our findings of adrenal dependent, stress-induced lymphopenia as well as adrenal independent effects on lymphocyte stimulation indicate that stress-induced modulation of immunity is a complex phenomenon involving several, if not multiple, mechanisms.

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8. Home-cage controls were housed four to a cage and not disturbed during the experimental period. Apparatus controls were placed in the shock apparatus with electrodes attached to their tails, but no current was applied. Rats in the two shock groups were similarly placed into the apparatus. Low-shock rats received 2-second shocks at variable intervals averaging one shock per minute. The current intensity was 0.8 mA for the first 8 hours, 1.0 mA for the second 8 hours, and 1.2 mA for the last 4 hours. High-shock animals were shocked on the same schedule but at higher intensity: 1.6 mA for the first 8 hours, 2.4 mA for the second 8 hours, and 3.0 mA for the final 4 hours.
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12. All animals from the shock and apparatus control groups were placed in the apparatus between 12 and 1 p.m. Home-cage control animals were left undisturbed. All of the rats were exsanguinated after the 20-hour shock period between 8 and 9 a.m. the following morning.
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19. Baseline lymphocyte numbers in the home-cage control animals were lower in the adrenalectomized group that received pellets than all other groups. This relative lymphopenia may be related to the higher baseline corticosterone concentrations in the adrenalectomized animals with steroid replacement (Fig. 1A).
20. Adrenal effects unrelated to stress will be described in a separate report.
21. Fisher's protected *t* single degree of freedom contrasts.
22. Radioactivity in the unstimulated cultures for each assay were as follows. For the isolated lymphocyte assay, the counts per minute in the unstimulated cultures across all operative groups were: home-cage control, 935 ± 164; apparatus control, 486 ± 54; low shock, 430 ± 83; high shock, 301 ± 50. For the whole blood assay: home-cage control, 399 ± 38; apparatus control, 209 ± 10; low shock, 184 ± 17; high shock, 189 ± 19. For the spleen cell assay, lower counts were found with the high shock animals only: home-cage control, 1242 ± 145; apparatus control, 1528 ± 187; low shock, 1367 ± 194; high shock, 786 ± 78. The unstimulated cultures in the isolated lymphocyte and whole blood assays were significantly suppressed by the stressful conditions, consistent with our previous study (*1*) in which stress effects were observed in unstimulated cultures.
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Coupling of Dopamine D_1 Recognition Sites with Adenylate Cyclase in Nuclei Accumbens and Caudatus of Schizophrenics

Abstract. *Sodium fluoride, guanylimidodiphosphate, and the D_1 dopamine receptor agonist SKF 38393 elicited a greater activation of adenylate cyclase in homogenates of caudate nucleus in schizophrenic than in nonschizophrenic subjects used as controls. Similarly, a greater activation of adenylate cyclase by sodium fluoride was observed in the nucleus accumbens of schizophrenics. These findings suggest that the coupling of dopamine D_1 recognition sites with adenylate cyclase is more efficient in the brain of the schizophrenic, presumably because of an increased affinity of the G/F protein for guanosine 5′-triphosphate.*

A hypothesis that has been popular for almost two decades holds that an increased activity of brain dopamine receptors is a neurochemical defect operative in the etiology of schizophrenia (*1–3*). The validity of this hypothesis rests on the finding that the administration of certain dopamine receptor agonists such as amphetamines (*4, 5*) or methylphenidate (*6*) can elicit or exacerbate psychotic symptoms, while dopamine receptor antagonists not only relieve the symptoms of schizophrenia but their potency is proportional to their dopamine receptor blocking activity (*7*). However, there is little direct support for the dopamine hypothesis in the etiology of schizophrenia. In fact, experiments directed to demonstrate a change in the regulation of brain dopamine function in schizophrenia have been inconclusive. Not only the content of dopamine and of its metabolites were found to be similar in various brain areas of schizophrenic (S) and nonschizophrenic (NS) subjects (*8*), but the measurement of dopamine turnover (*9*) and the dopamine-sensitive adenylate cyclase (*10*) in dopamine-rich areas of the brains of S and NS subjects failed to reveal any differences.

The reports supporting a role of dopamine in schizophrenia show that the density of dopamine recognition sites is increased in dopamine-rich areas of S brains (*11–13*). Since the number of dopamine recognition sites is also increased in crude synaptic membranes prepared from the brains of rats that received daily injections of neuroleptics for several weeks (*14*), it is uncertain whether the elevated density of dopamine recognition sites detected in S brains is related to the long-term treatment with neuroleptics or to schizophrenia (*15, 16*).

In order to ascertain whether there is an abnormal dopamine transmission in S brains, we have compared the biochemical properties and the pharmacological profile of the postsynaptic dopamine receptor in dopamine-rich areas of S and NS brains. It is generally agreed that in the corpus striatum, nucleus accumbens, and other dopamine-rich areas of the brain, the postsynaptic recognition sites for dopamine are functionally linked to adenylate cyclase (*17, 18*). This link appears to be regulated by an intrinsic membrane protein, termed G/F protein (*19*). The G/F protein consists of several subunits that have binding sites for guanosine triphosphate (GTP), guanosine diphosphate (GDP), and NaF (*20*). When the dopamine recognition sites are occu-

pied by an agonist that activates the adenylate cyclase, the GDP bound to the G/F protein is released, while the binding of GTP to this protein is facilitated (*21, 22*). This process is considered to be a rate-limiting step for adenylate cyclase activation by neurotransmitters (*22*).

In the present report the responsiveness of adenylate cyclase to dopamine receptor stimulation and the coupling efficiency of the G/F protein were evaluated by measuring basal, neurotransmitter-, NaF-, and guanylimidodiphosphate-stimulated adenylate cyclase activities in several brain regions of S and NS subjects (*23*). The brains were collected and dissected (*24*).

The psychiatric conditions of the various subjects, from whom brain tissues were used, were diagnosed from chart reviews using Research Diagnostic Criteria (*25*). The subjects were divided into two categories: S patients, including chronic paranoid schizophrenics and chronic undifferentiated schizophrenics, and NS subjects (Table 1). The basal activity of adenylate cyclase in homogenates of nucleus accumbens, nucleus caudatus, hippocampus, and cerebellar cortex was similar in both types of brains (Fig. 1, A and B). In homogenates of nucleus accumbens and caudatus the maximum stimulation of adenylate cyclase elicited by dopamine ($10^{-4}M$) was also similar. Moreover, the addition of serotonin ($10^{-4}M$) to homogenates of hippocampus and of isoproterenol ($10^{-4}M$) to homogenates of cerebellar cortex of S brains failed to stimulate adenylate cyclase to a greater extent than that observed in similar structures of NS brains. Comparison of a group of S subjects (A, B, D, and I in Table 1) whose blood plasma content of neuroleptic drugs was below the range of biochemical detection (< 1 nmole) with a group of S subjects (C, E, F, G, and H in Table 1) whose blood plasma contained measurable amounts of neuroleptics showed that the extent of adenylate cyclase stimulation elicited by dopamine in homogenates of nucleus accumbens and caudatus was similar and did not differ from the extent of stimulation seen in NS brains.

These observations suggest that in S subjects repeatedly treated with neuroleptics the possible presence of neuroleptics in the brain may not account for the lack of responsiveness of adenylate cyclase to stimulation by dopamine. Moreover, the extent of adenylate cyclase stimulation elicited by maximally effective concentrations of NaF or guanylimidodiphosphate was greater in homogenates of nucleus caudatus from S brains than in homogenates of the same area prepared from NS brains (Fig. 1A). In addition, maximum stimulation of adenylate cyclase by NaF was greater in homogenates of nucleus accumbens from S brains than in those from NS brains (Fig. 1B). No differences were found in the guanylimidodiphosphate and NaF stimulation of adenylate cyclase activities in homogenates of cerebellar cortex or hippocampus prepared from either S or NS brains (Fig. 1, A and B). It is of interest that in the nucleus accumbens or caudatus from brains of NS subjects treated repeatedly with neuroleptics (F and K), the extent of adenylate cyclase stimulation by NaF or guanylimidodiphosphate was comparable to that found in similar brain structures dissected from NS subjects who never received long-term treatment with neuroleptics. Four separate evaluations showed that the median effective dose of guanylimidodiphosphate for stimulating adenylate cyclase in homogenates of nuclei accumbens and caudatus of NS brains was 26 ± 3 and 56 ± 11 μM, respectively (D, F, L, and N) and 13 ± 1.5 and 8 ± 1 μM in homogenates of the respective brain structures dissected from S brains (D, H, I, and M).

The increased responsiveness of adenylate cyclase to NaF or guanylimidodiphosphate stimulation in S brains suggests that the mechanism that brings

Table 1. Autopsy data on coded S and NS subjects. See Fig. 1.

Code	Age	Sex	Hours from death to autopsy	Neuroleptics detectable in blood plasma	Cause of death
			NS subjects		
A	19	M	11.5	No	Car accident
B	19	M	21.5	No	Car accident
C	21	M	17.5	No	Suicide
D	24	M	19.5	No	Car accident
E	27	F	24	No	Car accident
F	29	F	18	Yes	Car accident
G	30	M	12.5	No	Homocide
H	32	M	14	No	Car accident
I	36	F	20.5	No	Suicide
J	44	M	6.5	No	Homicide
K	45	F	27.5	Yes	Homicide
L	46	M	10	No	Homicide
M	53	M	9.5	No	Cardiac infarction
N	68	M	7.5	No	Car accident
O	30	M	23	No	Pulmonary embolism
P	33	F	3	No	Fire
Q	44	M	3.5	No	Smoke inhalation
R	53	M	31	No	Cardiac infarction
S	81	M	?	No	Asthmatic attack
T	65	F	?	No	Undetermined
U	87	F	?	No	Pneumonia
V	81	M	6	No	Arteriosclerosis
W	83	M	6	No	Septicemia
X	69	M	22	No	Abdominal hemorrhage
			S subjects		
A	28	M	6.5	No	Suicide
B	21	M	14	No	Suicide
C	25	M	15	Yes	Suicide
D	30	M	20	No	Suicide
E	36	M	7	Yes	Hyponatremia
F	38	M	19	Yes	Car accident
G	44	F	19	Yes	Breast cancer
H	46	F	15	Yes	Car accident
I	46	M	3.5	No	Suicide
J	52	F	20	Yes	Suicide
K	58	F	11	Yes	Car accident
L	63	M	20	Yes	Suicide
M	68	F	11	Yes	Suicide
N	18	M	4	Yes	Suicide
O	30	M	3.5	No	Suicide
P	38	F	12	Yes	Undetermined
Q	53	M	?	Yes	Cancer
R	57	M	7	Yes	Cardiac infarction
S	58	M	14	Yes	Undetermined
T	81	F	?	Yes	Arteriosclerosis
U	78	M	9	Yes	Cardiac infarction
V	54	M	?	Yes	Cardiac infarction

about dopamine-mediated signal amplification of adenylate cyclase activity is more efficient in S than in NS brains. This contrasts with the reports of other investigators (*10*) and with the present results, which show a similarity in the extent of dopamine stimulation of adenylate cyclase in dopamine-rich areas in S and NS brains. It is likely that the difference in the stimulation of adenylate cyclase from S brains by dopamine guanylimidodiphosphate, and NaF could be due to differences in the organization of the various components of the dopamine receptor. When dopamine is used as a stimulant it has to interact with dopamine recognition sites; when the stimulant is NaF or guanylimidodiphosphate the transmitter recognition site is bypassed and these agents act directly on the G/F protein. In the brain two functional types of dopamine recognition sites have been described: the D_1 type mediating a stimulation and the D_2 type mediating an inhibition of adenylate cyclase activity (*26*). Both D_1 and D_2 recognition sites require a G/F protein for the coupling to adenylate cyclase (*27, 28*), and when these recognition sites are stimulated simultaneously the response of adenylate cyclase is equal to the algebraic summation of the response elicited by the simultaneous stimulation of stimulatory and inhibitory dopamine receptors (*26*). Moreover, the increased density of [^{3}H]spiroperidol binding sites in nuclei accumbens and caudatus described in S brains is primarily due to the increased density of D_2 recognition sites (*9*), while the number of D_1 recognition sites is not altered (*29*).

Table 2. Increased responsiveness of striatal adenylate cyclase to D_1 receptor stimulation in the brains of S subjects. Numbers in parentheses are numbers of subjects. Basal adenylate cyclase activity in the nucleus caudatus of NS subjects (subjects A through I and subject N) and S subjects (subjects B through G, I, J, and M through O) was 68 ± 2.7 and 76 ± 4.9 pmole of cyclic AMP per milligram of tissue per minute, respectively. SKF 38393 or dopamine ($5 \times 10^{-6}M$, $5 \times 10^{-5}M$, and $10^{-4}M$) were used to stimulate adenylate cyclase in homogenates. Maximum cyclase stimulation was obtained by $10^{-4}M$ of either agonist.

Group	Increase in cyclic AMP at maximum stimulation (pmole/mg tissue-min)	
	SKF 38393	Dopamine
NS	52 ± 4.0 (8)	48 ± 2.2 (9)
S	82 ± 6.4 (9)*	45 ± 3.0 (9)

*Significantly different from corresponding value for NS subjects ($P < 0.05$, Student's *t*-test).

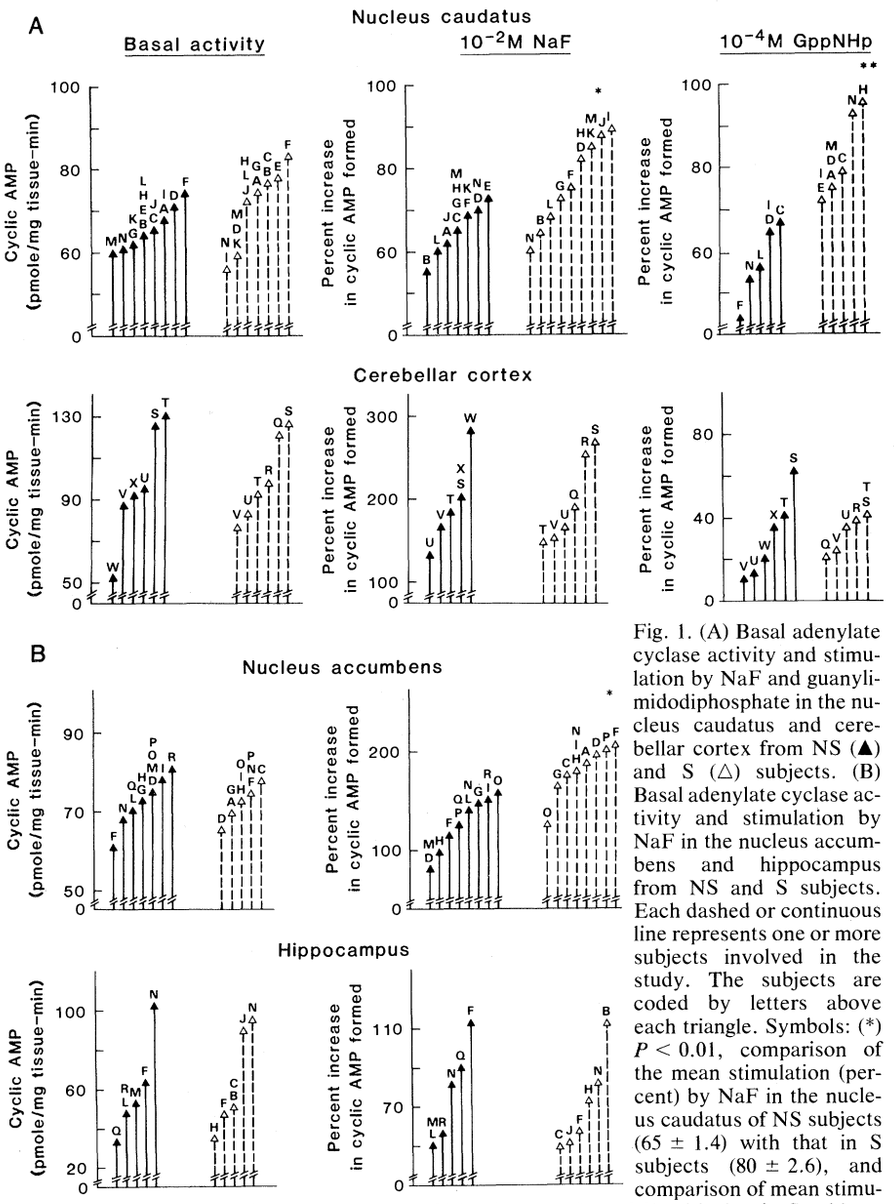

Fig. 1. (A) Basal adenylate cyclase activity and stimulation by NaF and guanylimidodiphosphate in the nucleus caudatus and cerebellar cortex from NS (▲) and S (△) subjects. (B) Basal adenylate cyclase activity and stimulation by NaF in the nucleus accumbens and hippocampus from NS and S subjects. Each dashed or continuous line represents one or more subjects involved in the study. The subjects are coded by letters above each triangle. Symbols: (*) $P < 0.01$, comparison of the mean stimulation (percent) by NaF in the nucleus caudatus of NS subjects (65 ± 1.4) with that in S subjects (80 ± 2.6), and comparison of mean stimulation by NaF in the nucleus accumbens of NS subjects (125 ± 5.7) with that in S subjects (179 ± 8.2). (**) $P < 0.01$, comparison of mean stimulation by guanylimidodiphosphate (*GppNHp*) in the nucleus caudatus of NS subjects (55 ± 4.5) with that in S subjects (84 ± 3.6).

It can be inferred that the inhibitory action on adenylate cyclase elicited by D_2 receptor stimulation masks the greater responsiveness of the enzyme to D_1 receptor stimulation. Perhaps such a masking effect is evident in the S brain. Since the number of binding sites for radioligands specific for D_1 receptors is similar in dopamine-rich areas of S and NS brains (*29*), it is possible that the dopamine recognition sites that are increased in S brains (*9*) are the type that opposes the D_1 receptor–mediated adenylate cyclase activation. The finding that, in homogenates of hippocampus and cerebellar cortex (two areas virtually without dopamine receptors), the responsiveness of adenylate cyclase to the stimulation of G/F protein by NaF or guanylimidodiphosphate is similar in S and NS subjects supports the view that

To ascertain whether there is a difference in the properties of D_1 and D_2 receptors in the S brain, we measured the stimulation of adenylate cyclase elicited by a specific agonist for D_1 receptors in homogenates of nucleus caudatus from S and NS brains. In nucleus caudatus of S brains the increment in the formation of adenosine 3′,5′-monophosphate (cyclic AMP) elicited by SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine), a selective D_1 receptor agonist (*26*), was significantly greater than in NS brains, while in homogenates of nucleus caudatus from S and NS brains dopamine elicited a similar increase in cyclic AMP formation (Table 2). This finding indicates that when the activation of adenylate cyclase in homogenates of dopamine-rich areas of S brains is tested with an agonist that acts simultaneously on D_1 and on D_2 receptors, the increased responsiveness of the S brain D_1 receptor cannot be detected.

in dopamine-rich areas of the S brain the activity of G/F protein may be increased or its coupling with adenylate cyclase facilitated.

The increase in responsiveness of the D_1 receptors located in the S brain could involve an increase in the number of G/F protein molecules operative in adenylate cyclase stimulation or an increase in the affinity of G/F protein for guanylimidodiphosphate. An increase in affinity is suggested by the observation that in homogenates of nucleus caudatus from S subjects the concentration of guanylimidodiphosphate needed to elicit half-maximum stimulation of adenylate cyclase is only 1/2 to 1/7 of that required by homogenates from nucleus caudatus of NS subjects. Such an increased affinity may cause a greater efficiency in the function of the G/F protein and in turn may facilitate the coupling of dopamine recognition sites with adenylate cyclase. In conclusion, the present data suggest that the enhanced dopamine function believed to be associated with schizophrenia may be the expression of an increased efficiency in the G/F coupling operative in linking D_1 recognition sites to adenylate cyclase.

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23. The dissected brain areas, which have been stored at −70°C, were gently homogenized in 10 volumes (weight to volume) of 10 m*M* tris-maleate buffer (*p*H 7.4) containing 2 m*M* EGTA by using glass-teflon homogenizers. Adenylate cyclase activity was determined in a portion of homogenate corresponding to 1 mg of fresh tissue. The incubation mixture contained 82.5 m*M* tris-maleate buffer (*p*H 7.4), 20 m*M* $MgSO_4$, 1 m*M* isobutylmethylxanthine, 0.5 m*M* EGTA, 0.05 m*M* GTP, and 5 m*M* adenosine triphosphate in a final volume of 500 μl. The incubation was carried out in the presence and absence of four different concentrations of NaF (1, 2.5, 5, and 10 m*M*) or guanylimidodiphosphate (5×10^{-6}, 10^{-5}, and 5×10^{-4}*M*) at 30°C for 5 minutes. The reaction was stopped by heating the samples at 90°C for 5 minutes. The amount of cyclic AMP formed was measured by radioimmunoassay (New England Nuclear).
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Modulation of the Metastatic Capability in B16 Melanoma by Cell Shape

Abstract. *The lung colonization of B16-F1 cells grown in flat and spherical configurations was studied. Cells cultivated in vitro as spheroids on a nonadhesive substrate expressed in a reversible fashion a marked increase in their propensity to establish metastases. The altered metastatic capability was accompanied by a reversible reduction in the accessibility of cell surface proteins to external iodination and by a dramatic decrease in the synthesis of vimentin.*

Metastasis is an active sequential process whereby the cells from the primary growth site invade the intravascular compartment and may attain once again an extravascular position at the target organ, where they proliferate to become metastases (*1*). Using metastatic cell variants exhibiting low, high, or moderate metastatic properties, we found in various tumor systems that highly metastatic cell variants differ from their mildly metastatic counterparts in their pattern of adhesion to the solid substrate as well as in their ability to establish cognitive interactions (*2, 3*). Since the hematogenous spread of tumor cells involves marked alterations in cell shape and morphology, we addressed the possible interrelation between cell shape–responsive growth control and the metastatic phenotype of tumor cells (*3*). The rationale for this approach stems from recent studies suggesting that the loss of cell shape–dependent growth control is a central feature in cell malignancy (*4*). In this study we attempted to determine whether the proliferation of cells under conditions of controlled cell shape may alter the metastatic phenotype of a specific cell line. We utilized the recently developed technology of preparing nonadhesive substrates by coating the culture plates with nontoxic transparent films of poly(2-hydroxyethylmethacrylate) [poly(HEMA)], which allows the control of cell shape (*5*). Growth under these conditions was used as a model system to mimic the different growth patterns obtained in vivo in the extravascular and intravascular compartments. We found that the growth of B16-F1 melanoma cells in vitro in a spherical configuration induced a marked increase in the metastatic capability of the cells in vivo and a faster spreading on tissue culture dishes, as compared to growth as a monolayer in a flat configuration. This altered biological behavior of the cells was accompanied by alterations in the accessibility of the cell surface proteins to external iodination and a dramatic decrease in vimentin biosynthesis. These alterations were reversible on reattachment and spreading, suggesting a central role for cell shape in the modulation of the metastatic capability.

B16-F1 melanoma cells from semiconfluent monolayers were harvested from cultures in their exponential growth phase with 2 m*M* EDTA in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS), *p*H 7.2. The cells were then washed and resuspended in PBS. Single-cell suspensions were seeded on control plates or on plates coated with 0.12 percent poly(HEMA) at a density of 10^5 cells per 60-mm plate. At various intervals thereafter the cells were harvested by adding EDTA (10 m*M*) to the growth medium for 5 minutes at 37°C. Gentle