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4. Two types of evidence suggest there is a seasonal change in the male EOD: (i) These fish appear to breed during the rainy season (early October), which correlates with the appearance of the mature male EOD. (ii) The EOD's of captive, mature males revert to the female-type waveform but are reinduced by testosterone. We did not castrate males with fully transformed EOD's since the EOD's of captive males revert to the female form and we did not have enough males to define a possible difference between the "normal" reversible condition and castration.
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8. Microelectrode studies show that action potentials of the posterior and anterior faces underlie, respectively, the EOD's positive and negative phases (*7*). We believe the stalk's potential can account for the inflection of an EOD's positive phase (Fig. 1d).
9. Electrocyte morphology also differs in these fishes.
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11. Electrode orientation or proximity (within 15 cm) does not affect the EOD waveform; only the amplitude changes.
12. We used a PDP8/e with Lab8/e A/D converter sampling at 24.83 μsec per point.
13. The Q-10 value was determined by measuring the EOD duration of two specimens of another sympatric species, *B. brachyistius* (triphasic), over the range of 18° to 25.8°C.
14. All significance values are derived from a least-squares fit to a straight line, two-tailed *t*-test for significance of regression coefficient. Post-testosterone-treated group: linear slope = − .03 msec/day, $t = 3.91$, significant at $P < .001$, d.f. = 22. Gonadectomized, testosterone-treated group: linear slope = .08 msec/day, $t = 3.84$, significant at $P < .01$, d.f. = 16. DHT-treated group: linear slope = .05 msec/day, $t = 3.67$, significant at $P < .01$, d.f. = 20.
15. The data for Fig. 2, b to e, could be fit to the equation: $y = y_0 + y_f(1 - e^{-t/\tau})$. The time constant, τ, for each curve is Fig. 2b: 7.8 days, [standard error (S.E.) = 3.4; d.f. = 76]; Fig. 2c: 9.3 (S.E. = 6.5, d.f. = 18); Fig. 2d: 2.7 (S.E. = 1.9, d.f. = 15); and Fig. 2e: 1.4 (S.E. = 0.8, d.f. = 19).
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Dopamine-β-Hydroxylase Activity and Homovanillic Acid in Spinal Fluid of Schizophrenics with Brain Atrophy

Abstract. *Schizophrenic patients with high ventricle brain ratios and cortical brain atrophy, as shown by computerized tomography, had decreased spinal fluid concentrations of homovanillic acid and dopamine-β-hydroxylase activity. These decreased cerebral spinal fluid concentrations in patients with brain atrophy support the proposal of disturbed noradrenaline and dopamine neurotransmission in a subgroup of schizophrenic patients.*

Brain atrophy has been detected in some schizophrenic patients by computerized tomography (CT) (*1–9*). Abnormalities in scans in schizophrenia have been associated with neuropsychological impairment (*2, 4–6*) and other nonpsychotic symptoms (*9*). Biochemical abnormalities in schizophrenics with brain atrophy have rarely been reported (*10*). Despite evidence of a dopamine disorder in schizophrenia (*11*), spinal fluid concentrations of the dopamine metabolite

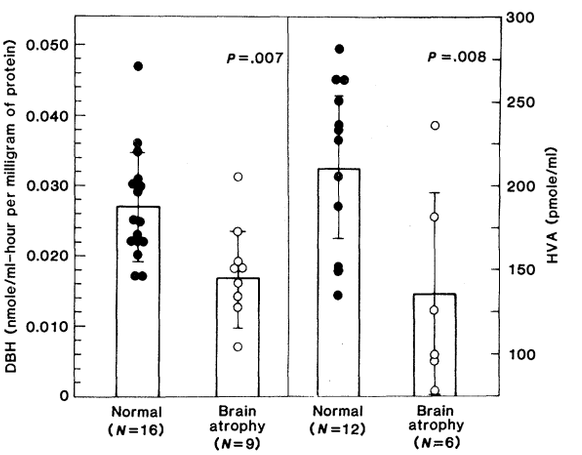

Fig. 1. Specific DBH activity and HVA content in the spinal fluid of patients with normal CT scans or brain atrophy. The probability given is that associated with the *t*-test for comparing two means (see Table 1). Error bars represent 1 standard deviation.

Table 1. Relation of normal and abnormal CT scans and VBR and the presence or absence of cortical atrophy to three variables in the spinal fluid of drug-free schizophrenic patients. The DBH activity and HVA concentrations were significantly lower in patients with brain atrophy. Values are mean + S.E.M. The probabilities are those associated with the *t* statistic for the difference between normal and abnormal. N.S., not significant.

Status	DBH					HVA		
	Patients (*N*)	Specific activity: nmole/ml-hour per mg protein	*P*	Activity: nmole/ml-hour	*P*	Patients (*N*)	pmole/ml	*P*
CT scans								
Normal	16	0.027 ± .0020	.007	1.05 ± .170	.107	12	214 ± 13.5	.008
Abnormal	9	0.018 ± .0023		0.58 ± .006		6	138 ± 23.8	
VBR								
Normal	20	0.026 ± .0017	.005	0.98 ± .139	.0056	16	191 ± 15.0	
Abnormal	5	0.014 ± .0021		0.49 ± .075		2	167 ± 67.2	
Cortical atrophy								
Absent	17	0.025 ± .0022	N.S.	0.99 ± .171	.029	17	203 ± 14.5	.09
Present	7	0.019 ± .0024		0.60 ± .055		7	145 ± 23.3	

homovanillic acid (HVA) have not consistently shown statistically significant differences between schizophrenics and other psychiatric and nonpsychiatric comparison groups (*12*). Dopamine-β-hydroxylase (DBH), which catalyzes the conversion of dopamine into norepinephrine in noradrenergic neurons, may be a marker of noradrenergic neurons. Stein and Wise proposed that chronic schizophrenic patients have decreased brain DBH activity because an endogenous neurotoxin destroys noradrenergic neurons (*13*). Low activity in cerebrospinal fluid (CSF) (*14*) and in brains (*15*) of schizophrenics postmortem has not been consistently reported. Because schizophrenia is thought to be a heterogeneous disorder, presence or absence of brain atrophy may explain the inconsistent results. We compared spinal fluid concentrations of HVA and DBH activity in schizophrenic patients with normal and abnormal CT scans and found decreased spinal fluid concentrations of HVA and decreased DBH activity in those with abnormal scans.

Physically healthy schizophrenic patients (N = 33, mean age 26, standard deviation 7.7 years, range 18 to 53 years) voluntarily participated in these studies at the Clinical Center of the National Institutes of Health after giving written informed consent. They were diagnosed by two psychiatrists who used the DSM III criteria. Patients with a history of chronic drug abuse, alcoholism, or other possible causes of brain atrophy were excluded. The CT scans, made on an EMI-1010, yielded 12 radiographic images (160° by 160° matrix; without contrast) of different brain levels from which ventricle size [ventricle brain ratio (VBR)] (*7, 16*) and measures of atrophy of cortical fissures and sulci (*3, 17*) were measured. Patients with readings more than 2 standard deviations above the mean of the VBR measurements of a control population (> 8.3 percent; N = 6) or with abnormal sulci (N = 8) were placed in the abnormal CT scan group (N = 11). Others were considered normal (N = 22). Three patients had only an abnormal VBR, five showed only sulcal atrophy, and three had both abnormalities.

For all patients, placebo capsules were substituted for medication an average of 33 days (range 14 to 76 days) before lumbar punctures. Patients were also kept on similar low monoamine, alcohol-free, and caffeine-restricted diets, and spinal fluid was collected under rigidly standardized conditions (*18*). DBH activity was measured in the CSF of 25 patients by a radio enzymatic method (*13, 19*). Spinal fluid concentrations of HVA and 5-hydroxyindoleacetic acid were measured in 18 patients by high-performance liquid chromotography with electrochemical detection (*20*) in a 1-ml portion from the first 12-ml pool.

Table 2. Relation of VBR and cortical atrophy to three variables in spinal fluid of drug-free schizophrenic patients. Cortical atrophy in one patient in the DBH group and in two patients in the HVA group could not be measured because of poor demarcation of the cortical areas. The probabilities are those associated with the t statistic for correlation coefficients. N.S., not significant.

Variable	VBR			Cortical atrophy		
	r	P	Patients (N)	r	P	Patients (N)
DBH (nmole/ml-hour per mg protein)	−.52	.007	25	−.23	N.S.	24
DBH (nmole/ml-hour)	−.56	.004	25	−.14	N.S.	24
HVA (pmole/ml)	−.57	.01	18	−.49	.05	16

The HVA concentrations and DBH activity in CSF of the patients with abnormal scans were lower than those of patients with normal scans (Fig. 1 and Table 1). The negative correlations between VBR and the CSF variables were statistically significant (Fig. 2 and Table 2). In the patients with normal CT scans, VBR and HVA concentrations were correlated significantly (r = −.56, P = .055, N = 12) (*21*). A possible effect of spinal fluid dilution cannot be excluded, although spinal fluid protein concentrations were identical in the two groups.

The association between abnormal CT scans and decreased spinal fluid HVA content and DBH activity may well reflect disturbed dopamine metabolism and loss of noradrenergic neurons. Mettler postulated a relation between schizophrenia and a disturbance of the basal ganglia (*22*), the caudate nucleus of which contributes most of the HVA to the spinal fluid. Therefore, low HVA content in CSF reflects decreased dopamine metabolism in the basal ganglia, and may be related to dopamine receptor supersensitivity (*23, 24*). Increased brain HVA content has not been reported (*25*), but Crow *et al.* (*24*) observed decreased HVA in the caudate nucleus as Bowers (*26*) did in CSF. Buchsbaum *et al.* (*27*), using positron emission tomography, found a statistically significant decrease in brain metabolism in both the frontal cortex and the left basal ganglia of a small group of schizophrenic patients with normal VBR's. Decreased HVA concentrations in patients with cortical atrophy thus suggest an impairment of the corticostriatal pathways that may affect dopamine metabolism in the basal ganglia. Furthermore, the frontal cortex is the primary projection area of mesocortical dopamine neurons. If enlarged VBR's are associated with loss of other than dopamine cells in the caudate nucleus, compensatory mechanisms may lead occasionally to increased HVA concentrations. One patient with a large VBR (13.5 percent) and minimal cortical atrophy had a high concentration of HVA (234 pmole/ml) (Fig. 2). This compensatory normalization of dopamine activity has also been found in patients with Huntington's disease and in rats with striatal kainic acid lesions (*28*).

Spinal fluid DBH concentrations below the mean were found in patients with abnormal CT scans but also in a few with normal scans, suggesting that different mechanisms may decrease DBH activity. The activity of DBH in spinal fluid correlates with that of 3-methoxy-4-hydroxyphenylglycol in normal subjects but not in schizophrenic patients (*29*), which suggests a disturbance in the regu-

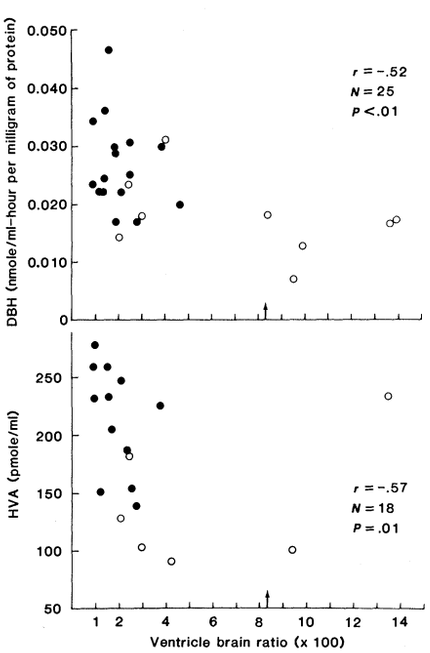

Fig. 2. The relation of ventricle brain ratio and DBH and HVA in spinal fluid. Symbols: ○, values associated with brain atrophy; ●, absence of cortical atrophy; and ↑, cutoff for abnormal VBR (> 8.3 percent).

latory mechanisms of DBH or norepinephrine activity. Our amphetamine studies in schizophrenics (*30*) indicated a state dependent dysregulation of the dopamine system. As in drug-induced DBH inhibition, genetically low DBH activity may lead to dopamine efflux from noradrenergic neurons, whereas low DBH activity due to cell loss will lead to decreased norepinephrine input upon the dopamine system (*31*).

Chronic treatment with neuroleptics does not explain brain atrophy. Some elderly and chronically treated schizophrenic patients have small VBR's (*8, 32*), and large VBR's have been observed in patients with schizophreniform psychosis, which predates the diagnosis of schizophrenia and extensive pharmacotherapy. We did not find a correlation of CT measures with increased duration of illness or months of hospitalization, suggesting that atrophy occurred some time before the onset of the illness and is not progressive. Environmental factors (*9, 32, 33*) such as perinatal damage (*34*) or viral infections (*35, 36*) may have caused brain atrophy.

We did not find a relation between poor premorbid adjustment (*37*) and CT abnormalities. Nasrallah *et al.* (*8*) observed that patients with paranoid schizophrenia, who have in general a better premorbid functioning, had high VBR's. In contrast, Weinberger *et al.* (*5, 38*) found that severely ill, chronic schizophrenic patients with high VBR's had the worst premorbid adjustment and did not respond to neuroleptics. As did Rieder *et al.* (*2*), we observed that some patients with abnormal CT scans had an episodic illness that responded to neuroleptics. These discrepancies in clinical findings may be due to sample composition. The patients studied by Weinberger *et al.* (*5, 38*) were much more severely ill and treatment-resistant and remained hospitalized after the study.

Abnormalities in monoamine metabolism associated with brain atrophy are not specific to schizophrenia but have been observed in patients with degenerative neurologic disorders such as Alzheimer's and Huntington's diseases (*39*). In other words, abnormalities in CT scans are probably not associated with the psychotic aspects of the illness, but more with neuropsychological impairment (*2, 4, 6, 9*) and the vulnerability to develop a schizophrenic syndrome. We suggest that the division of schizophrenia into dopamine-related and non–dopamine-related schizophrenia on the basis of evidence from CT scans and neuroleptic response is premature. The specificity of enlarged VBR's or cortical atrophy associated with decreased spinal fluid DBH activity and HVA concentrations is not known, but the findings are consistent with proposals of a basal ganglia disturbance and noradrenergic cell loss in some schizophrenic patients.

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16. The scan with the longest ventricles (through the body of the lateral ventricles) was selected for each subject; the images were randomly mixed and the names covered during measurement. The area of the lateral ventricles and of the intracranial space of each subject was measured with a compensating polar planimeter by two raters (L.S.M. and D.R.W.) with an intraclass correlation, .92; a mean of the two ratings was computed. VBR was calculated by dividing the ventricular area by the intracranial area and multiplying by 100.
17. Two raters (L.S.M. and R.O.R.) independently rated the overall prominence of the sulci (interhemispheric, Sylvian, and other fissures) on Rieder's 0 to 3 scale (*2*), which is now widely accepted (*32*). Reliability between raters was high (intraclass correlation .95). The raters' average score was used for cortical atrophy. In three patients cortical atrophy could not be measured because cortical markings were not sharp.
18. Patients fasted and had bedrest the night before lumbar punctures, which were made between 8:30 and 9:00 a.m. with the patient in lateral decubitus position. The CSF was collected in polypropylene tubes and put on ice immediately. The portion for DBH assay was frozen on dry ice within 30 minutes and then stored in liquid nitrogen (−196°C). All punctures were nontraumatic by cell count, and to diminish further the risk of plasma contamination, we studied DBH activity from a pooled sample (0.5 ml) of the 16th to the 28th milliliter of CSF. Specimens were assayed twice within 3 days of the lumbar puncture with a modification (*15*) of the radioenzymatic method of P. B. Molinoff, R. Weinshilboum, and J. Axelrod [*J. Pharmacol. Exp. Ther.* **178**, 425 (1971)]. No significant endogenous inhibition of DBH in the CSF was noted. The coefficient of variation was 8 percent within runs and 13 percent between runs. Recovery of added DBH was always greater than 95 percent. Data analysis was done for DBH activity with and without correction for protein concentration in the CSF because a significant correlation between CSF protein and DBH activity was observed ($r = .87$, $N = 33$, $P < .0001$).
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40. We thank the patients and nursing staff under S. Beal, head nurse of the 4-East Clinical Research unit, for their cooperation; P. Lerner for radioenzymatic assay for DBH, and D. R. Weinberger for training L.S.M. to read the VBR.

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Interaction with Membrane Remnants of Target Myotubes Maintains Transmitter Sensitivity of Cultured Neurons

Abstract. *Parasympathetic neurons, when cultured alone, lose sensitivity to acetylcholine, but if striated muscle is included in the culture, neuronal chemosensitivity is maintained. The membrane remnants of myotubes ruptured by osmotic shock also supported the responsiveness of the cultured neurons to transmitter, whereas muscle-conditioned medium or membrane remnants of nonmuscle embryonic skin cells did not support this responsiveness. The regulation of chemosensitivity by contact of neurons with the target cell membrane may be important in the formation and maintenance of neuronal circuitry.*

The maintenance of appropriate chemosensitivity by neurons is a prerequisite for adequate function of the nervous system. Although cell culture may allow the controlled study of the cellular processes that govern neuronal chemosensitivity, considerable variation in the sensitivity of cultured ganglionic neurons to transmitters and in the binding of labeled neurotoxins has been observed (*1, 2*). Thus, in one study (*3*), the formation of functional synapses between cultured ciliary ganglion neurons was not detected because of the loss of neuronal chemosensitivity in cultures lacking target myotubes, but in another study (*4*), synaptic activity was found in many neurons from similar cultures. When myotubes were included in the cultures along with the neurons, adequate neuronal chemosensitivity (*3*) and evidence of interneuronal transmission were more frequently found (*3, 4*). The present study indicates that specific interactions among the elements of a culture may determine neuronal responsiveness to transmitter [see also (*2*)]. Contact of the neurons even with the membrane remnants of myotubes sustains for at least 1 week the retention of active transmitter receptors.

Neurons of the chick ciliary ganglion were removed from embryos at 10 to 12 days of incubation, dissociated, and plated (*5*). The culture substrate was one of the following. (i) An air-dried layer of rat tail collagen. The neurons grow in the presence of ganglionic nonneural cells, but muscle tissue is absent. (ii) A monolayer of chick pectoral myotubes plated as myoblasts 1 week earlier and allowed to fuse. An immediately available target tissue is presented to the neurons; neuromuscular junctions are formed rapidly and are maintained over several weeks of culture (*3, 6*). (iii) "Muscle material"—a culture substrate composed of myotube membranes. Myotube cultures were lysed in sterile water for 1 to 2 hours before the addition of the suspension of embryonic neurons. Examination of this substrate under Hoffman-modulated optics or phase-contrast optics shows no live tissue, but the outlines of empty cell and myotube remnants are clearly visible (Fig. 1D). (iv) "Fibroblast material"—a culture of epithelial or fibroblast cells uncontaminated by myoblasts (*7*). Subsequent lysis with water leaves a nonmuscle membrane-material substrate.

Table 1. Sensitivity of neurons to iontophoretically applied ACh. Numbers in parentheses indicate numbers of neurons tested. Each neuron was tested for a response to a pulse (2 to 50 msec) of ACh at a minimum of three separate sites on the somal surface. For all culture conditions, except culture on myotubes and myotube material, 55 percent of the 54 neurons tested were sensitive to the transmitter at 2 to 4 days in vitro. However, the level of sensitivity was often low (5 to 50 mV per nanocoulomb).

Culture medium	Percentage of neurons responding at	
	6 to 8 days	> 12 days
Collagen	0 (32)	
Myotubes	100 (26)	100 (30)
Myotube material	88 (51)	0 (7)
Fibroblast material	0 (44)	
Collagen in muscle-conditioned medium*	0 (15)	
Collagen in fibroblast-conditioned medium*	0 (7)	

*Medium was conditioned for 24 hours (*3*).

Neuronal chemosensitivity was monitored by intracellular recording of the cultured neurons during iontophoresis of acetylcholine (ACh) with high-resistance (150 to 300 megohm) micropipettes (*3, 5*). Acetylcholine is the major transmitter for the ciliary ganglion, and chemical transmission through the ganglion begins before the stage of development attained by neurons used to establish the cultures in the present study (*8*). Therefore, in all cases, if recordings are taken 1 or 2 days after the cultures are seeded with neurons, the iontophoretic application of ACh results in a depolarization (*3*). This depolarization is accompanied by a drop in membrane input resistance and is completely blocked by the ACh antagonist *d*-tubocurarine (*3*). Eserine, an inhibitor of acetylcholinesterase, causes an increased amplitude and duration of the depolarization (Fig. 1C). The reversal potential for the response to ACh, estimated by extrapolation (Fig. 1B), in five cells was −10 to −22 mV, similar to published values for synaptic and iontophoretic ACh potentials (*9*). In addition, delivery of ACh pulses at frequencies higher than 2 Hz caused rapid desensitization, whereas the response was unchanged if several seconds elapsed between pulses. Therefore, the neuronal response to ACh is most likely mediated by specific receptors, activation of which results in an increase in membrane permeability to ions and thus a depolarization from the normal resting transmembrane potential.

No response to ACh was seen from neurons grown without muscle if the recordings were taken at six or more days after plating [Table 1 and (*3*)], and the percentage of responsive neurons progressively declined between 2 and 6 days in vitro. This confirms that the loss of active receptors during the first week of culture of neurons plated alone is responsible for the transmission failure of interneuronal synapses (*3*). In contrast, neurons plated onto myotubes retain active receptors for several weeks in vitro [Table 1 and (*3*)]. Acetylcholine sensitivity at the most responsive mem-