of the three shaker channels. Their outputs were recorded and averaged over 32 cycles for each of the 36 stimuli used. The amplitudes and axis angles of the stimuli were calculated from these waveforms.

17. An optical displacement transducer (MTI KD320 Fotonic sensor) was used to confirm that the cylinder moved in phase and as a rigid body at 140 Hz and that the fish's skull, clamped to the cylinder wall, moved at the same phase and amplitude.
18. All data were collected in the form of period histograms for stimulus durations of 1 second. For cells with low spontaneous rates the tracking variable was the first harmonic discrete Fourier transform of the period histogram, R_1, with the criterion set at 80 spikes per second [D. O. Kim and C. Molnar, *J. Neurophysiol.* **52**, 16 (1979)]. For spontaneous cells, the tracking variable was the coefficient of synchronization (R_1 normalized to spike rate), with a criterion equal to 0.5. The threshold was tracked as follows. A stimulus was presented at an intermediate attenuation value and increased or decreased in 10-dB steps until the criterion response was bracketed. A preliminary threshold was then estimated by linear interpolation. Stimuli were then presented 3 dB above and 3 dB below the threshold estimate, and a final threshold estimated from these two points by linear interpolation (or extrapolation).
19. The phase angle of the period histogram is essentially the location in the cycle of the peak spike rate.
20. The threshold points of Fig. 1 were converted to Cartesian coordinates and a least-squares method used to find the best fitting line, omitting the highest threshold from the analysis.
21. Each best-fitting threshold line has intercepts on the abscissa and ordinate. Since a line was defined in each of the *x-y*, *x-z*, and *y-z* planes, there were two estimates each of the *x*, *y*, and *z* axis intercepts. These two estimates were averaged for each of the three intercepts, and these three points were used to define the equation for the three-dimensional threshold plane.
22. Spherical coordinates consist of an azimuth (straight ahead defined as 90°), an elevation (vertical defined as 0°), and a magnitude (defined as an acceleration in decibels with reference to 1 g).
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Neuroleptic-Induced Decrease in Plasma Homovanillic Acid and Antipsychotic Activity in Schizophrenic Patients

Abstract. *Plasma-free homovanillic acid, a major metabolite of dopamine, was measured in chronically ill schizophrenic patients both before and during treatment with the antipsychotic phenothiazine, fluphenazine. Neuroleptic treatment was associated with a significant time-dependent decrease in plasma homovanillic acid from pretreatment values, which were significantly elevated when compared with those of age- and sex-matched healthy control subjects. Further, both the absolute concentrations as well as the neuroleptic-induced reductions in plasma homovanillic acid determined over 5 weeks of neuroleptic treatment were statistically significantly correlated with ratings of psychosis and improvement in psychosis, respectively. These findings suggest that the delayed effects of neuroleptic agents on presynaptic dopamine activity may more closely parallel their therapeutic actions than do their immediate effects in blocking postsynaptic dopamine receptors and that a decrease in dopamine "turnover" may be responsible for their antipsychotic effects.*

The dopamine hypothesis of schizophrenia proposes that a functional overactivity of central dopaminergic neurotransmission underlies the development of psychotic symptoms (*1*). The evidence for this hypothesis derives principally from pharmacological studies in animals, in which neuroleptics (i) cause a short-term increase in dopamine turnover by blocking postsynaptic dopaminergic receptors (*2*), (ii) bind to dopaminergic receptors of the D_2 subtype with affinities that are correlated with their clinical potencies as antipsychotics (*3*), and (iii) selectively increase (on short-term administration) (*4*) and then decrease (on long-term administration) the spontaneous firing rate of dopamine-containing neurons in the substantia nigra and ventral tegmental areas of the rat (*5*). The dopamine hypothesis, however, has received only inconsistent support from clinical studies carried out in schizophrenic patients themselves, in which dopamine metabolites have been measured in body fluids such as cerebrospinal fluid (CSF) or in postmortem brain tissue (*6*). Further, administration of drugs that enhance functional dopamine activity in the brain have been reported not only to worsen schizophrenic symptoms (*7*) but in some cases to improve (*8*) them. More recently, reports of brain atrophy in some patients with schizophrenia have suggested the possibility of two forms of schizophrenia, one involving dopaminergic pathophysiology and the other structural brain damage of unknown etiology (*9, 10*).

Perhaps the most difficult observation to reconcile with the dopamine hypothesis of neuroleptic action is that short-term administration of antipsychotic drugs blocks dopamine receptors in animals including humans, whereas their therapeutic effects require more prolonged administration, usually a minimum of 3 weeks (*11*). Since there is now evidence that fluctuations in the plasma concentrations of the major dopamine metabolite homovanillic acid (HVA) parallel changes in dopamine turnover in the CNS (*12*), we carried out a longitudinal study in which we measured plasma HVA in chronically ill schizophrenic patients both before and during treatment with the potent neuroleptic, fluphenazine. We now report that fluphenazine treatment was associated with a time-dependent decrease in plasma HVA concentration from pretreatment values, which were significantly elevated when compared with age- and sex-matched normal controls. Further, the decreases in plasma HVA concentration were statistically significantly correlated with clinical improvement, indicating that the therapeutic effects of antipsychotic drugs like fluphenazine, may follow from a decrease in dopamine turnover that is secondary to dopamine receptor blockade.

Eight patients (three male, five female; age range, 20 to 29) meeting DSM III criteria (*13*) for the diagnosis of schizophrenia and free of physical illness were studied on a psychiatric research ward of the National Institute of Mental Health. After giving informed consent to participate in the study, all patients were kept on a low-monoamine, low-alcohol, and caffeine-restricted diet. Neuroleptic administration was carried out according to double-blind placebo-controlled methods. After a minimum of 14 days on placebo (mean ± standard error of the mean medication-free days; 28 ± 6 days) the antipsychotic phenothiazine, fluphenazine, was substituted and administered in doses ranging between 20 and 60 mg/day (*14*). Behavioral ratings were obtained weekly by physicians and daily by specially trained nursing staff, none of whom were aware of the medication status of the patient (*15*). Blood was collected by venipuncture in EDTA-containing tubes 3 days per week between 0730 and 0930 (*16*). After an overnight fast, patients remained on restricted activity until blood collection was complete. Plasma, obtained within 30 minutes of collection by centrifuging whole blood (800*g*, 10 minutes), was stored at −20°C. Plasma-free HVA was assayed by high pressure liquid chromatography with electrochemical detection (*17*).

Fluphenazine administration resulted in a time-dependent decrease in the plasma concentration of HVA (Fig. 1). The decrease in plasma HVA relative to pre-

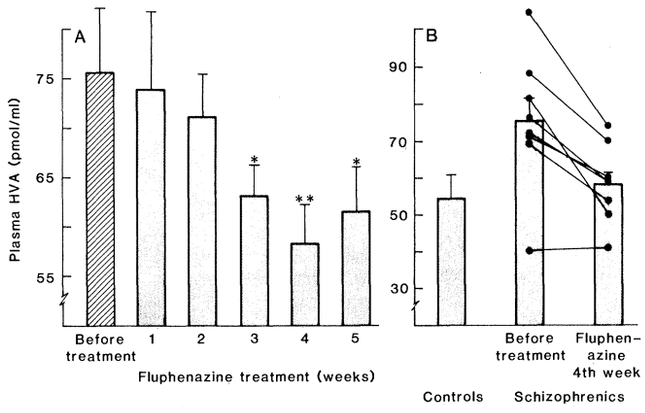

Fig. 1. The effects of fluphenazine treatment on plasma HVA in schizophrenic patients. (A) Mean (± standard error) of weekly plasma HVA values for the group (n = 8) were significantly decreased during treatment with fluphenazine [repeated measures $F(5, 35)$ $P < 0.001$]. Plasma HVA concentrations at weeks 3, 4, and 5 of fluphenazine treatment were significantly lower than those during the last medication-free (pretreatment) week (Tukey's multiple comparison test: $*P < 0.05$; $**P < 0.01$). (B) A comparison of plasma HVA concentrations in schizophrenic patients (n = 8) before and during fluphenazine treatment (4th week) with those of unmedicated, healthy controls (n = 8) (*22*). The pretreatment plasma HVA concentrations in patients during the last medication-free week were significantly greater than those of age- and sex-matched controls [$t(14) = 2.30$, $P < 0.05$]. The values for schizophrenic patients and healthy controls represent the mean of three and two determinations, respectively, during the same week. Plasma HVA concentrations during week 4 of fluphenazine treatment were significantly lower than during the last pretreatment week [paired $t(7) = 4.54$, $P < 0.01$], but did not significantly differ from those of normal controls.

treatment concentrations reached statistical significance during weeks 3, 4, and 5 of neuroleptic treatment (Fig. 1A), a time course which paralleled the improvement in clinical state (Fig. 2A). The possible relation between plasma HVA concentration and the change in clinical state during neuroleptic treatment was further examined by correlating plasma HVA concentrations with psychosis ratings. Mean weekly concentrations of plasma HVA were significantly correlated to weekly ratings of global psychosis by physicians (r = 0.82, $P < 0.05$). The relation between plasma HVA and psychosis is further supported by two correlations observed when plasma HVA concentrations from all time points were considered: the absolute concentration of plasma HVA was correlated with nurses' ratings of psychosis (r = 0.82, $P < 0.001$) (Fig. 2B), and the change in plasma HVA from pretreatment (baseline) values during fluphenazine administration was also correlated with the change in nurses' ratings (r = 0.87, $P < 0.001$) (Fig. 2C) (*18*).

Since physical activity increases the concentration of plasma HVA (*19*), consideration was given to possible effects of sedation produced by fluphenazine. We observed a significant decrease in plasma HVA only relatively late in drug treatment (weeks 3, 4, and 5), and thus the decrease was observed at a time when the sedative effects of neuroleptics are typically diminishing. A relation was also found between plasma HVA concentration and physicians' BPRS ratings of withdrawal-retardation (*15*) (r = 0.77, $P < 0.10$). Thus, higher plasma HVA concentrations tended to be associated with greater motor retardation and social withdrawal. Although the latter group of so-called "negative" symptoms has been considered to be relatively refractory to neuroleptic treatment (*9, 20*), our experience, as well as that of others,

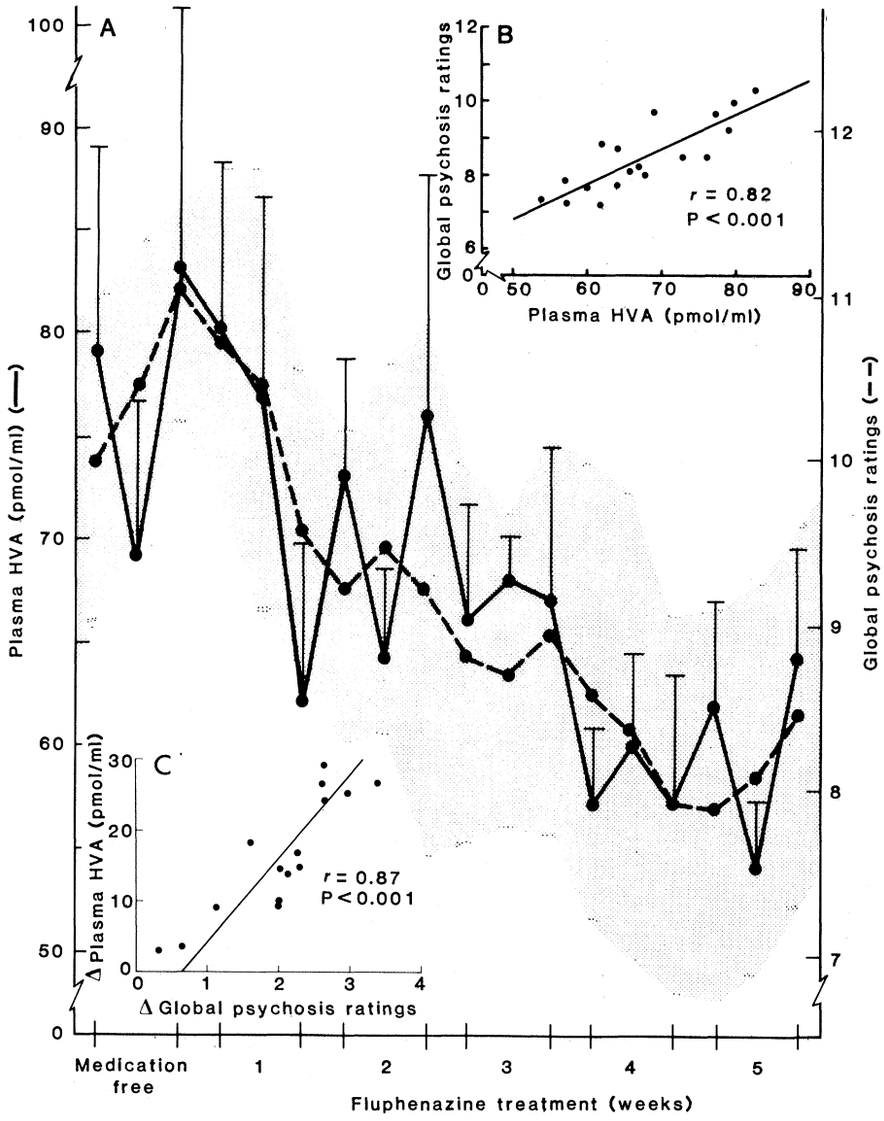

Fig. 2. Correlation between plasma HVA and psychosis in schizophrenic patients before and during treatment with fluphenazine. (A) Each data point represents the mean (± standard error) of plasma HVA values (●—●) or the corresponding nurses' ratings of psychosis (●---●) (*15*) for all patients (n = 8) obtained during the last medication-free week and continuing throughout 5 weeks of treatment with fluphenazine (*14, 18*). Values for psychosis ratings represent the nurses' ratings over the period beginning 12 hours before and 12 hours after each plasma determination. Vertical bars and shaded area represent standard errors of the means for plasma HVA and psychosis ratings, respectively. (B) Correlation between plasma HVA concentration and psychosis over all time points (n = 18). (C) Correlation between the change in plasma HVA and the change in psychosis during fluphenazine treatment (n = 15).

suggests that in many patients these symptoms occur concurrently with positive symptoms such as delusions and hallucinations, and are at least partially responsive to neuroleptic treatment (*21*).

In related experiments, we also compared the plasma concentrations of HVA in schizophrenic patients before and during treatment with fluphenazine with those of medication-free normal controls. Eight healthy volunteers, matched by sex and by age to within 2 years of the schizophrenic patients and free of any medical or psychiatric illness were studied on two separate days during the same week (*22*). Blood drawings separated by at least 2 days were carried out identically to those in the patient group. The mean pretreatment plasma HVA in the schizophrenic patients was significantly greater than that of the group of healthy controls [$t(14) = 2.30$, $P < 0.05$] (Fig. 1B). Further, the decrease in plasma HVA associated with long-term fluphenazine treatment resulted in plasma HVA concentrations that were not significantly different from those of the control group.

Although it is not clear whether the fluphenazine-induced decrease in plasma HVA observed in our patients reflects a decrease in the turnover of central or peripheral dopamine (or both), the contribution of HVA derived from the brain to that circulating in plasma has been estimated to be substantial, approximately 40 to 50 percent (*12*). Intraventricular injection of 6-hydroxydopamine to rats, for example, results in a 40 percent decrease in plasma HVA, whereas bilateral electrothermic lesions of the nigrostriatal dopamine pathway, alone, decrease plasma HVA by 25 percent (*23*). Further, both neuroleptic administration (*24*) and electrical stimulation of the nigrostriatal dopamine pathway (*23*), which increase brain dopamine tunover and HVA concentration, also increase plasma HVA in monkeys and rats.

Adaptation to the neuroleptic-induced increase in dopamine turnover during prolonged neuroleptic administration has been demonstrated with electro-physiologic techniques as well as from biochemical measures such as brain HVA concentration (*25*) and tyrosine hydroxylase activity (*26*). In studies of psychiatric patients, neuroleptic treatment has been shown to increase HVA in the CSF (*27*). The development of "tolerance" to this effect has also been described (*28*), although reports indicate variability in its occurrence between individual patients (*29*). Although in our group of patients, as a whole, fluphenazine did not increase concentrations of plasma HVA, in five of the eight patients increases in plasma HVA above baseline were observed during the first week of treatment (not shown). The three patients in whom we did not see an initial increase in plasma HVA had the highest pretreatment values; furthermore, the only patient in whom fluphenazine did not eventually decrease plasma HVA had the lowest pretreatment value.

Our finding of a time-dependent decrease in plasma HVA during neuroleptic treatment in schizophrenic patients demonstrates an adaptational change in dopamine release consistent with changes in the firing rate of dopaminergic neurons previously reported in animal studies (*5*) and links this pattern to the therapeutic effects of neuroleptic treatment in schizophrenic patients. In rats, the decrease in the number of spontaneously active dopaminergic neurons in the substantia nigra and ventral tegmental areas seen during long-term neuroleptic administration is believed to be related to depolarization inactivation (*5*). Thus, this overdepolarization of dopaminergic neurons observed during long-term neuroleptic administration results in an overall decrease in dopamine-related neuronal activity. That plasma HVA concentrations in our patients were reduced to, but not below, those of healthy controls, may imply that neuroleptic-induced depolarization inactivation, if it occurs in humans, may reduce the number of spontaneously active dopaminergic neurons toward that of normal individuals. The elevated plasma concentrations of HVA observed in our patients before treatment may indicate an increased central turnover of dopamine in schizophrenia or in a subgroup of patients with schizophrenia, as has been suggested by findings associating a good response to neuroleptics with low CSF concentrations of dopamine β-hydroxylase, the enzyme that catalyzes the synthesis of norepinephrine from dopamine (*30*). However, it is also possible that the increase in plasma HVA may be a result of discontinuing neuroleptic treatment. Regardless of the actual mechanisms responsible for the elevated plasma HVA in unmedicated schizophrenic patients, the reduction in plasma HVA, and presumably dopamine turnover, produced by neuroleptic administration was significantly correlated with a decrease in psychotic symptoms. These data, therefore, support the hypothesis that the mechanism of action of neuroleptics, such as fluphenazine, involves a decrease in dopamine turnover and that monitoring changes in plasma HVA may be a useful tool in assessing the antipsychotic activity of pharmacologic agents.

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14. An attempt was made to maintain patients free from all medications for 4 weeks before beginning fluphenazine treatment. In three of the

eight patients, behavioral disturbances were so severe that fluphenazine administration was begun after 14 medication-free days. There was no relationship between the number of medication-free days and either the initial concentration of plasma HVA or baseline psychosis ratings. Fluphenazine was administered orally at 10 mg/day and was increased to a final daily dose on the basis of individual clinical state by the end of the first week. This dose (mean ± standard error of the mean, 36 ± 6 mg/day) was maintained whenever possible throughout the remaining 4 weeks of the study. In each patient, benztropine was added during the first week (dose range, 0.5 to 2.0 mg) to reduce extrapyramidal side effects.

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18. A maximum of 18 possible blood collections were possible: three pretreatment samples followed by three samples for each of five treatment weeks. For these analyses, if a patient missed an individual blood collection, the corresponding clinical rating for that day was excluded from group means for that time point. In all cases, group means for plasma HVA and psychosis ratings during drug treatment were less than at baseline; thus, changes in HVA and psychosis ratings represent a reduction in plasma HVA and clinical improvement, respectively. Means and standard errors of individual patients' variation coefficients for any of the 6 weeks of study ranged from 5.44 ± 1.13 percent to 11.2 ± 3.0 percent; for all weeks, 7.2 ± 0.83 percent. For the correlative analysis we used conservative degrees of freedom based on two-thirds of the number to minimize the effects of repeated inclusion of individuals [M. H. Quenouille, *Associated Measurements* (Academic Press, New York, 1952); p. 170].
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Albumin and Australian Frogs: Molecular Data a Challenge to Speciation Model

Abstract. *Vertebrate speciation in the southwest of Australia has long been viewed as resulting from multiple invasions of eastern source stocks during the Pleistocene. Microcomplement fixation studies of serum albumin evolution in frogs of the genus* Heleioporus *provide the first hard data on age and phylogenetic relationships among species in this genus and lead to rejection of the multiple invasion model in favor of speciation occurring in Western Australia. The albumin molecular clock was used to estimate that the species divergences in this genus occurred between 4 million to 12 million years ago in the late Tertiary (Pliocene-Miocene), rather than in the Quaternary (the last 2 million years).*

Ideas on the biogeography of southern Australia have been influenced by allopatric speciation models that were based on isolation by deserts or marine barriers. In Western Australia the absence of the major geographic barriers presumed essential for allopatric speciation led Main *et al.* (*1*) to propose a model of repeated invasions of source stocks from eastern Australia to account for the high species diversity in several genera of frogs in Western Australia. This "multiple invasion hypothesis," which White (*2*) recently described as "ingenious, even if completely imaginary," has been widely adopted to explain species diversity in many vertebrate and invertebrate taxa (*3*).

For the past quarter of a century this multiple invasion hypothesis has enjoyed widespread popularity, in part because of its general applicability to so many taxonomic groups, but also because previously (*4*) there have not been new data available to test objectively the validity of this model.

Initially, the hypothesis suggested that there were two source stocks per frog genus in eastern Australia and that frog stocks were dispersed from east to west across the continent during Pleistocene glaciations. During interglacials, gene pools were disrupted, and the eastern stock and its western cognate populations diverged. Main *et al.* (*1*) recognized up to three migration and differentiation cycles. The model was based on information on the structure of male advertisement calls, the results of artificial hybridization studies, information on morphology, and knowledge of the breeding ecology of frogs in three genera: *Heleioporus*, *Neobatrachus*, and *Crinia*. Although criticized (*2, 5*), the model was never tested directly (*4*). An adequate test requires (i) independent evaluation of the phylogenetic relationships of the species under consideration, and (ii) an estimate of the timing of lineage divergence events. Microcomplement fixation studies of the evolution of serum albumin can provide these important data (*6*).

Albumin was obtained from serum preserved in phenoxyethanol (*7*) for all six species of *Heleioporus*. Established procedures (*8*) were used for the purifi-

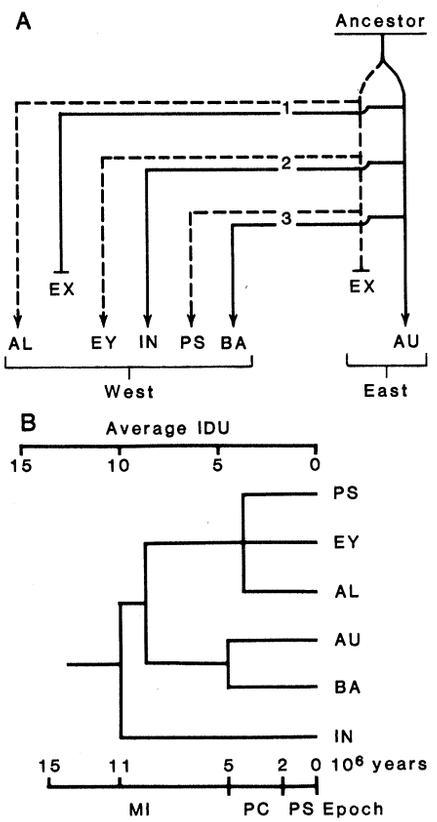

Fig. 1. (A) Predicted relationships in the genus *Heleioporus*; the dashed lines represent descendants of an arid adapted form, solid lines descendants of a related mesic adapted form [after Lee (*10*)]. Numbers 1, 2, and 3 refer to migrations across Australia, with 1 the oldest, 3 most recent. EX, extinct; AU, *H. australiacus*; PS, *H. psammophilus*; EY, *H. eyrei*; AL, *H. albopunctatus*; BA, *H. barycragus*; IN, *H. inornatus*. (B) Albumin relationships among *Heleioporus*. On the basis of MC'F analysis, the estimated immunological distance between any two species is the sum of the horizontal linkages. The vertical distances between species have no evolutionary significance. PS, Pleistocene; PC, Pliocene; and MI, Miocene epochs.