metrically homologous. The dilatations along axis 1-5 presumably increase the space available for the forebrain at the same time the jaw is shrinking. The dilatations along axis 2-4 represent constant expansion of the hind braincase in the direction causing the least increase in moment about the cranial base. Axis 3-6 seems aligned closely with the main axis of the temporalis muscle in modern man; it may represent the preservation of a fairly constant mechanical advantage for that muscle. As the jaw shortens and the back of the head lengthens, the constancy of this axis ensures the constant relation of temporalis pull to the condyle, mandibular notch, and temporomandibular joint (*9*).

This analysis cannot prove that the forms I have invoked *are* in the hominid line. It shows only the rigorous geometric consistency of such a sequence, but it does not explain how the axes are maintained by selection. It is possible that there exist only a few axes along which evolutionary change, specifically size allometry and neoteny, can take place, so that change of form itself is canalized.

Further research with biorthogonal grids should include (i) certain computational improvements—better treatment of singularities, inclusion of data inside the boundaries, extension to solid forms; (ii) application to morphology and systematics in other suitable taxa; and (iii) application to problems of growing form in embryology, orthodontics, and the like. The advantage of the method proposed is great. Measurement of shape change independent of shape itself allows us to construct just those curves along which shape is most significantly varying, instead of having to guess at the appropriate morphometric index in advance.

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Liquid Chromatographic Analysis of Endogenous Catecholamine Released from Brain Slices

Abstract. *A simple new liquid chromatographic technique has been applied to transmitter release studies in brain slice preparations. This method, which gives direct readings of picomoles of endogenous transmitter released, has been shown to yield reliable results with a variety of brain slice manipulations.*

Brain slices in oxygenated physiological buffers retain the metabolic and electrical characteristics of neuronal tissue (*1*, *2*), and such preparations are widely used to study in vitro release of neurotransmitters (*3*–*6*). Traditionally, to obtain the requisite sensitivity for release studies, the slices are loaded by incubation with radiolabeled transmitter. We now report an extremely sensitive and simple method for quantitatively determining the release of endogenous catecholamine transmitters and their metabolites from brain slices. The new technique, by measuring endogenous release, avoids one of the sources of uncertainty in radiolabeling methods—namely, redistribution. For instance, in tissues which contain several transmitter systems, such as hypothalamus, uptake of labeled amines may not be specific to a single neuron type. It is unlikely that endogenous amines redistribute, and hence the new technique may be more specific.

The essence of the method is to utilize a cell slice chamber of small volume, 1 ml or less, and to couple this with the extreme sensitivity of high-performance liquid chromatography (LC) with electrochemical detection. The latter requires only 5- to 10-μl samples of the physiological buffer for analyses. The tissue is equilibrated in the buffer chamber, and then micro portions are removed to give a measure of spontaneous release. Drugs are next added in a 5- to 10-μl volume, or electrical stimulation is applied. After sufficient release time, portions of the fluid are again removed for analysis of induced (stimulated) release. All samples are injected directly on the LC column, and no pretreatment is necessary. Quantitative results are obtained by comparison with standard calibration plots. A complete description of the LC equipment and an assay for norepinephrine (NE) and dopamine (DA) with quantitative results in the picomole region have recently appeared (*7*). [None of the sample manipulations for brain tissue described for the assay in (*7*) are required in the usage described here; direct injection of samples is sufficient.]

Tissue slices were taken from Sprague-Dawley rats weighing approximately 350 g. After the rats were decapitated the brain was removed, rinsed with cold Yamamoto (*8*) or Krebs-Ringer bicarbonate (*8*) buffer, and placed on its dorsal surface on a glass plate over ice. Specific sections were removed by standard dissection procedures, weighed, and cut with a sharp razor blade into slices about 0.5 to 1.0 mm thick. The average weights of the hypothalamus and a single striatum were 43.7 and 42.1 mg, respectively.

Any style cell slice chamber may be used, although the volume must be altered to accommodate 1 ml or less of solution. In the work described here, a volume of 200 μl was used in a glass micro test tube with a built-in oxygen bubbler and platinum stimulating electrodes. The cell was immersed in a water bath at 37°C. A convenient chamber for simultaneous electrophysiological and chemical studies has been described by Spencer *et al.* (*9*) and could be miniaturized for LC sampling.

The minced tissue was transferred to the chamber and allowed to equilibrate. After 15 minutes, duplicate or triplicate

10-μl samples were withdrawn with a Hamilton syringe and injected into the chromatograph to get a mean value for the spontaneous release. A 10-μl portion of *dl*-amphetamine solution was then added to the chamber to give a final concentration between 5×10^{-9} and $5 \times 10^{-5}M$. Fifteen minutes later other 10-μl samples were taken for analysis. Whenever samples were removed, equivalent volumes of buffer were put back to keep the cell volume constant, which simplified concentration calculations. Table 1 shows spontaneous and amphetamine-induced release of DA from striatal tissue. These data agree well with those from previous studies (*10*). Amphetamine concentrations lower than $5 \times 10^{-7}M$ were ineffective in causing release.

In this technique, as opposed to a perfusion method, the released transmitters continue to accumulate in the chamber with time. For any particular study, fixed times should be chosen and maintained for sampling spontaneous and induced releases. For example, in all the experiments reported here, samples were taken 15 minutes after the tissue was placed in the chamber to measure spontaneous release. Immediately thereafter the chosen drug or stimulus was applied, and samples were taken after another 15 minutes to measure induced release. Checks revealed that the accumulation effect of spontaneous release is small. Successive spontaneous release values were measured at 15 and 30 minutes, and the second value was only about 2.5 percent greater than the first.

Hypothalamic tissues provide an interesting test of the method since NE and DA are present in a ratio of approximately 5 to 1 and the LC method can simultaneously analyze both compounds. Both catecholamines are released after *dl*-amphetamine is added to the tissue chamber.

For studies of release by electrical stimulation, a 20-mg portion of corpus striatum was again placed in the tissue chamber to establish the spontaneous release. A Grass S44 stimulator with a constant-current attachment was used to provide a 1-minute train of 10-hertz, 20-msec pulses at 20 ma. Samples were again withdrawn and analyzed. Dopamine concentrations rose approximately 15 percent after stimulation and decayed away within 1 hour. These results show a slightly higher level of release than that reported by Farnebo and Hamberger (*11*).

Fresh striatal slices were incubated in $5 \times 10^{-6}M$ apomorphine or chlorpromazine for 30 minutes to study the effects of the uptake and accumulation of these drugs on later electrically induced release. The slices were then rinsed with fresh buffer and transferred to the tissue chamber. Electrical stimulation was initiated as described above. Figure 1 is a comparison of DA release between treated and untreated slices. Apomorphine considerably decreased stimulation-induced release, and chlorpromazine increased it by about 21 percent. These results were expected in light of the suspected actions of apomorphine and chlorpromazine on DA receptors, although variable effects have been reported with different concentrations of chlorpromazine (*12*).

Table 1. Spontaneous and amphetamine-induced release of endogenous dopamine from striatal slices. Each value in columns 1 and 3 is the mean ± standard error of the mean for three separate experiments, in each of which triplicate chromatographic samples were analyzed for each value. Tissue sample weights varied between 32.1 and 49.8 mg. Amphetamine-induced release = total amphetamine-induced release − spontaneous release. Percentage of total DA released = (mean induced release/total DA) × 100. The total DA content was determined by sonicating pools of striatal tissue ($N = 10$) in 0.1M perchloric acid and assaying by standard LC tissue analysis (*7*). Total DA = 10,710 ng per gram of striatal tissue = 70 pmole/mg. The standard deviation in these analyses was ± 6.0 percent.

Spontaneous DA release (pmole/mg)	Amphetamine (M)	Amphetamine-induced DA release (pmole/mg)	DA released by amphetamine (% of total)
3.49 ± 0.09	5.0×10^{-5}	32.40 ± 1.63	46.3
2.85 ± 0.19	5.0×10^{-6}	10.33 ± 1.13	14.8
3.22 ± 0.14	5.0×10^{-7}	2.45 ± 0.27	3.5
2.60 ± 0.06	5.0×10^{-8}	0.101 ± 0.01	
2.41 ± 0.16	5.0×10^{-9}	0.019 ± 0.00	

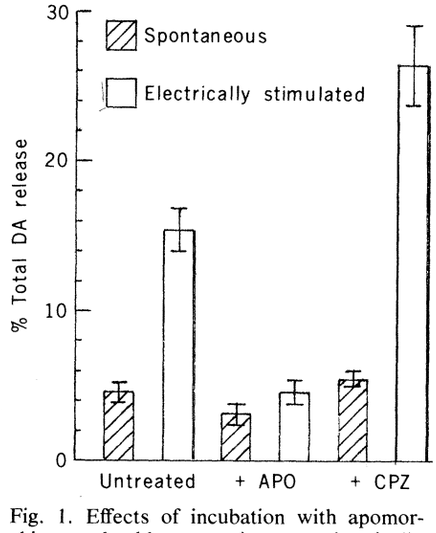

Fig. 1. Effects of incubation with apomorphine and chlorpromazine on electrically stimulated release of endogenous DA from striatal slices. (Shaded bars) Spontaneous release as percentage of total DA (100 × picomoles released/total picomoles of DA); (clear bars) electrically stimulated release less spontaneous release expressed as percentage of total DA. Total DA is 70 pmole per milligram of striatal tissue (see Table 1). Untreated indicates that tissue was not incubated; +APO that tissue was incubated with apomorphine; and +CPZ that tissue was incubated with chlorpromazine (see text for details).

The experiments reported here were designed only to illustrate that the LC analysis technique is a reliable and desirable new approach to studies of catecholamine release from brain slices. The results are comparable to those obtained with other sensitive analytical methods but require less expense and time. Of special interest is the fact that the LC method gives actual amounts (for example, picomoles) of released endogenous catecholamines. Both DA and NE can be studied simultaneously. These experiments did not even begin to utilize the full sensitivity of the LC method with electrochemical detection. Smaller preparations such as synaptosome beds could easily be studied. Coupling the LC method with previous incubation with a radioactive tracer is an obvious extension, and may allow differentiation of catecholamine release from various storage pools. We recently became aware of studies in which endogenous release was measured in a nonperfused cell chamber by use of radioenzymatic assays (*13*).

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Psoralen-DNA Photoreaction: Controlled Production of Mono- and Diadducts with Nanosecond Ultraviolet Laser Pulses

Abstract. *Samples of DNA which contain adducts of a new psoralen derivative, but no cross-links, have been prepared by irradiating mixtures of DNA and the derivative with single, 15-nanosecond pulses of laser light. Succeeding pulses introduce cross-links. The ability to rapidly and selectively create monoadducts and cross-links may allow the use of psoralens as probes of dynamic processes in living cells.*

Psoralens are linear tricyclic heterocycles which can intercalate between the base pairs of double-stranded nucleic acids, forming covalent adducts to pyrimidine bases upon absorption of long-wave ultraviolet light. If there is a second pyrimidine adjacent to a psoralen-pyrimidine monoadduct and on the opposite strand, absorption of a second photon can lead to formation of a diadduct which functions as an interstrand cross-link. This property has made psoralens useful probes of the secondary structure of DNA, either in isolated form (*1*, *2*) or in biological structures such as chromatin (*3*).

It is advantageous to be able to selectively create monoadducts, as distinguished from cross-links, for certain biological investigations. For example, monoaddition to DNA in vivo may well be a mutagenic event, but attempts to assay for mutagenicity of trimethylpsoralen have been complicated by the high lethality of the cross-linking which normally accompanies monoadduct formation. Moreover, rapid monoaddition of psoralens to replicating or transcribing DNA could label regions of the DNA which are accessible to intercalation and photoreaction without interfering with strand separation, and perhaps without blocking the action of polymerases or other functional entities. Labeled regions could be located later by purifying the DNA, exhaustively irradiating it to convert as many adducts as possible to cross-links, and then locating the cross-links by electron microscopy of denatured spreads of the DNA.

Two approaches are possible for the exclusive production of monoadducts. One is to use a very low light dosage during the photoreaction, so that it becomes highly unlikely that any single psoralen molecule would receive the two successive photons apparently necessary for cross-link formation. The difficulty with this procedure is that the concentration of monoadducts one can obtain is quite low. The other approach is to present the light in such a short pulse that there is no time for a second photon to be absorbed. It is believed that an excited triplet state is an essential intermediate for the photoreaction (*4*); thus, if the rate-limiting step is reaction from the triplet state, the light pulse need only be short compared to the triplet lifetime. We have utilized a frequency-doubled (347 nm), *Q*-switched ruby laser having a pulse length of approximately 15 nsec to produce exclusively monoadducts in this manner. A potential bonus of using such short irradiation times is the possible use of psoralens as fast probes of molecular dy-

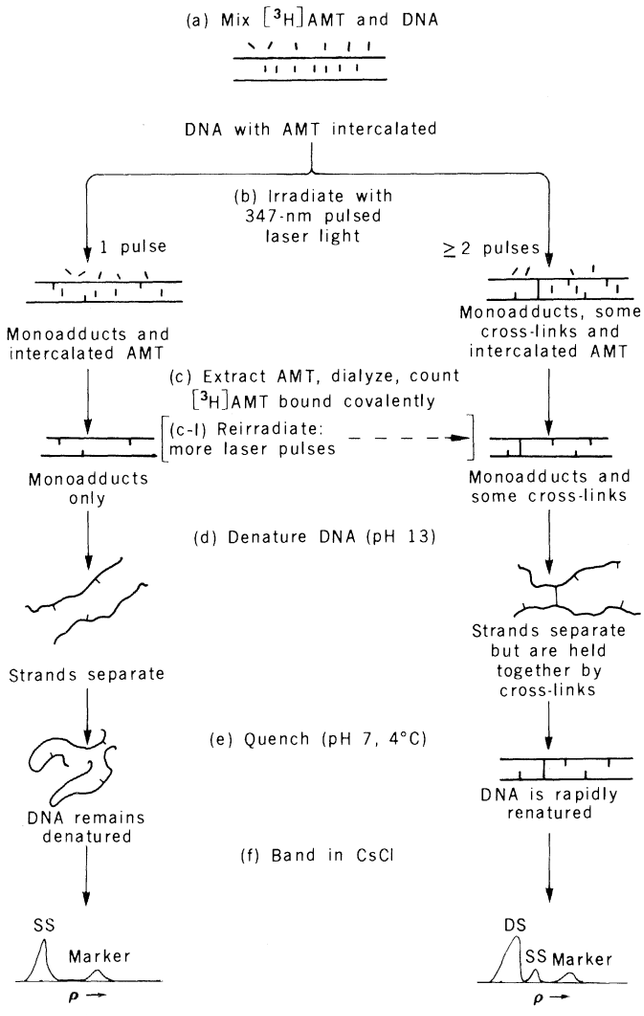

Fig. 1. Experimental scheme. (a) The T4 DNA was isolated by the procedure of Bautz and Dunn (*7*); AMT had a specific tritium activity of 1.42×10^5 count/min per microgram. Typically, we irradiated a 0.5- or 1.0-ml cylindrical quartz cell (path length, 1 or 2 cm) containing 50 μg of DNA and 5.6 μg of AMT per milliliter of a buffer solution containing 0.01*M* sodium phosphate (*p*H 7.0) and 1 m*M* EDTA (b). The beam almost completely filled the cell, although there were regions of greater and lesser intensity in the cross section. To average out these effects, the sample was mixed between successive pulses whenever more than one pulse was applied. The pulse energy was 50 to 100 mjoule. Free AMT was removed by making the sample 1*M* in NaCl and extracting two or three times with phenol (saturated with the above buffer), followed by dialysis through Spectrapore membranes against phosphate-EDTA buffer 1*M* in NaCl until a control sample which was unirradiated showed no residual tritium (c). The DNA concentration was then determined by ultraviolet absorbance at 260 nm, and 0.5 to 1.0 μg of DNA was mixed with 1 μg of *Micrococcus lysodeikticus* DNA (used as a buoyant density marker, ρ_{native} = 1.733 g/cm^3). The mixture was brought to *p*H 13 with NaOH (d), allowed to stand for 10 minutes at room temperature (22°C), and then cooled to 4°C, whereupon it was neutralized by addition of 0.2*M* NaH_2PO_4 (e). A 0.5-ml portion of CsCl was then added to give a final solution density of 1.720 g/cm^3. These samples were spun at 42,000 rev/min in a Beckman model E analytical ultracentrifuge equipped with an ultraviolet scanner (f), using double-sector titanium centerpieces. Abbreviations: *SS*, single-stranded; *DS*, double-stranded.