tion (*11*). After continuous administration of Δ^9-THC, however, the initial tachycardia was followed in some subjects by bradycardia. In this group of subjects the mean pulse rate was 60 per minute for the first 5 days (placebo period), rose to 66 per minute on the first day of cannabis administration, and dropped to a low of 54.3 per minute after 12 days of drug administration. Other cardiovascular effects consistent with Δ^9-THC intoxication (*12*) included a fall in mean systolic blood pressure of 14 mm-Hg, a fall in mean diastolic blood pressure of 17 mm-Hg, and an associated weight gain which averaged 4.54 kg per subject during the first 16 days of drug administration. The weight gain, due to fluid retention, was lost within 48 hours of stopping drug administration. Typical marijuana effects were also noted by changes in electroencephalograms, autonomic nervous system, perceptual motor tasks, endocrine system functions, and ward behavior. The participants reported subjective feelings of intoxication during the period of drug administration. Other experiments with similar subjects have shown that Δ^9-THC given orally produces a near complete cross-tolerance to single acute doses of smoked marijuana administered at various points during the oral dosage schedule (*12*).

An additional seven chronic marijuana smokers were studied. This group consisted of males and females aged 20 to 26 years. The group averaged 4.7 marijuana cigarettes per week with a mean duration of smoking of 4.6 years. The blood samples were drawn 8 to 72 hours after the last use of marijuana. Controls consisted of non-drug-using laboratory personnel in the same age range. Control and experimental lymphocytes were cultured at the same time. As indicated in Fig. 2, the mean PHA responses of marijuana smokers fell well within the normal range. These results are in agreement with those found in the hospitalized group and with those of a similar study reported by White and co-workers (*4*).

In summary, in otherwise healthy chronic marijuana smokers, eight of whom were observed under controlled conditions and given pharmacologic amounts of Δ^9-THC, normal lymphocyte responses to PHA were observed.

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10. From the nine controls remaining after extraction of the first six, a second group of six was randomly selected and compared to the first control group. There were no statistically significant differences between the control groups, and in comparisons with the subject, no difference was found whether the first or second control group was used.
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Aspartate: Distinct Receptors on *Aplysia* Neurons

Abstract. Aplysia *neurons have specific aspartate receptors that are distinct from those to glutamate. In some cells, aspartate selectively increases the membrane permeability to chloride, giving rise to a hyperpolarization, while on other cells it increases the permeability to sodium, causing a depolarization. There are also specific receptors for* L-*glutamate which mediate sodium, chloride, or potassium conductance increases, and another class of receptors activated by both glutamate and aspartate.*

While L-glutamate has been regarded as a putative excitatory neurotransmitter in both vertebrates and invertebrates, L-aspartate has been reported only to act like glutamate (*1, 2*). However, recent experiments in lobster muscle and the mammalian central nervous system have suggested that aspartate may have a distinct role as a neurotransmitter (*3*). Both aspartate and glutamate are involved in a number of cellular processes such as metabolism, protein formation, and intracellular osmotic and ionic regulation. Aspartate has been found to be a major anion in excitatory, inhibitory, and sensory nerve tissues in the lobster and crab (*4*).

In the gastropod mollusk *Aplysia*, glutamate (*5, 6*) and aspartate (*6*) have been detected in both ganglia and single nerve cells. Glutamate is uniformly distributed, while aspartate concentrations vary over a fourfold range among individual neurons. In contrast, hemolymph, muscle, and connective tissue have significantly lower concentrations. In a number of gastropod mollusks, aspartate has been reported to act like glutamate, which can cause hyperpolarizing or depolarizing responses, or both (*2, 7, 8*). Whether aspartate should be considered a putative neurotransmitter depends not only on its presence in nervous tissue and its ability to cause voltage and conductance changes, but also on the demonstration of aspartate receptors that are specific and distinct from those for other amino acids. We have found specific receptors for aspartate which evoke either depolarizing or hyperpolarizing responses.

Abdominal, cerebral, pleural, and buccal ganglia of *Aplysia californica* or *Aplysia dactylomela* were removed from the animal and pinned to a Sylgard (Dow Corning) layer in a Lucite chamber and perfused with artificial seawater containing 150 m*M* Mg^{2+} at room temperature (20° to 24°C). The connective tissue capsule was slit with a razor blade to expose the cell bodies. Neurons were penetrated with two independent micropipettes filled with 2*M* potassium acetate and having resistances of about 10 megohms. Recording was performed as before (*9*); one pipette was used to record membrane potential and the other to pass current pulses or d-c current for measurement of membrane resistance and reversal potentials. Drugs were applied by iontophoresis with a five-barreled extracellular micropipette. The iontophoretic control unit was designed to pass the same total charge through each of the five barrels, and would automatically vary pulse duration to achieve constant total charge (*10*). L-Glutamic acid and L-aspartic acid were dissolved in distilled water at concentrations of 1*M* and titrated to *p*H 8 with NaOH. Both were passed as anions, while acetylcholine chloride (ACh), 2*M*, was also iontophoresed as a cation. The resistance of each drug barrel was more than 20 megohms. When there was any question of the specificity of the iontophoretic response, two controls were included: (i) reversing the

Table 1. Times to peak and types of ions in responses to aspartate and glutamate. Values shown are means ± the standard error; the numbers of responses are shown in parentheses.

Type of response*	Response (seconds)		
	L-Glutamate	L-Aspartate	Acetylcholine†
Fast (Na^+) depolarizing	2.3 ± 0.4 (8)	2.2 ± 0.4 (8)	1.8 ± 0.2 (36)
Fast (Cl^-) hyperpolarizing	2.5 ± 0.4 (12)	2.5 ± 0.3 (12)	2.4 ± 0.2 (13)
Slow (K^+) hyperpolarizing	15.2 ± 1.93(5)		12.7 ± 1.7(6)

*Measured as conductance increase. †These data are taken from (*16*).

polarity of the iontophoretic pulse to test that the effect seen was not due to a direct effect of current flow across the membrane, and (ii) bath perfusion of aspartate and glutamate (10^{-5} to $10^{-4}M$) to verify the existence of distinct receptors. Since the experiments were performed in high Mg^{2+} seawater, responses to iontophoretic drug application may be considered a direct effect on receptors present on a single neuron and not due to activation of other cells (*11*).

Responses to aspartate were recorded in cells from all ganglia studied, although not all cells responded to either aspartate or to glutamate. Separate and distinct responses to aspartate were obtained in only a small percentage of the neurons. These were occasionally found in the abdominal ganglia, but were more frequent in the buccal ganglia on small neurons near the large cells B1 and B2 (*12*). Receptors for both aspartate and glutamate, as for glutamate receptors in *Onchidium* and *Helix* (*13*), were found only in the neuropil and not on the somata. This may have led to an underestimation of the percentage of neurons containing separate aspartate receptors, since it was necessary to search blindly for sensitive areas in the neuropil.

Iontophoretic application of aspartate, glutamate, and acetylcholine in four different neurons produced responses that were accompanied by a decrease in membrane resistance which was not due to membrane rectification. The neuron shown in Fig. 1A has three distinct responses, each resulting from different ionic conductances. A pulse of aspartate resulted in a depolarization of resting membrane potential which was abolished in Na^+-substituted seawater, where $tris^+$ was used in place of Na^+. Both glutamate and ACh caused hyperpolarizations. The response to glutamate was reversed in polarity below −60 mv (E_{Cl}^-), and was abolished in Cl^--free seawater where Cl^- was replaced by acetate. The ACh response remained unchanged in Cl^--free seawater and reversed in polarity below −80 mv (E_{K^+}). Thus, the aspartate response was due to an increase in Na^+ permeability, the glutamate response to an increase in Cl^- permeability, and the ACh response to an increase in K^+ permeability [see (*14*)]. In Fig. 1B, another cell responded to aspartate with a hyperpolarization of 6 mv, which was identified as an increase in Cl^- permeability, while glutamate had no effect. Figure 1C shows the converse situation where a neuron responded to glutamate but not to aspartate. In Fig. 1D, hyperpolarizations to both aspartate and glutamate were recorded in a single cell. Both of these responses were due to an increase in Cl^- permeability and could be cross-desensitized, suggesting that they result from activation of a common receptor. Likewise, there are common depolarizing responses due to an increase in Na^+ permeability. No cross-desensitization has ever been seen between the ACh response and responses to aspartate or to glutamate. Biphasic (Na^+ and Cl^-) responses have also been seen on application of both aspartate or glutamate and Cl^- and K^+ responses on application of glutamate [see (*13*)].

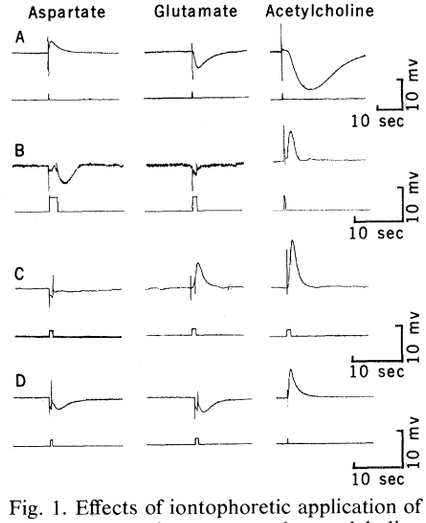

Fig. 1. Effects of iontophoretic application of L-aspartate, L-glutamate, and acetylcholine (ACh) on four different neurons from the abdominal (A and D) and buccal (B and C) ganglia. The upper recording in each trace is from the intracellular micropipette, while the lower trace shows the duration of the iontophoretic pulse. The cell in (A) had a resting membrane potential (RMP) of −50 mv, and iontophoretic currents were (in nanocoulombs): aspartate, 50; glutamate, 50; and ACh, 50. In (B), the cell had a RMP of −48 mv and currents were (in nanocoulombs): aspartate, 400; glutamate, 400; and ACh, 100. No response was seen with bath application of glutamate ($10^{-4}M$). In (C), the cell had an RMP of −45 mv and currents were (in nanocoulombs): aspartate, 500; glutamate, 200; and ACh, 200. No response was seen with a bath application of aspartate ($10^{-4}M$). In (D), the RMP of the cell was −52 mv and currents were (in nanocoulombs): aspartate, 400; glutamate, 400; and ACh, 200.

Table 1 summarizes the ionic conductances and the average times to the peaks of the responses of receptors to aspartate and glutamate as compared to acetylcholine. Neurons were unselected except for the requirement of a response to the selected drug and a clear identification of the ionic conductance involved, by changing seawater composition and by obtaining an inversion potential at levels close to those previously reported (−60 mv for E_{Cl}^-, −80 mv for E_{K^+}) (*14*). As for receptors to ACh, serotonin, and dopamine in *Aplysia* (*14–16*), there are specific depolarizing responses to aspartate and to glutamate due to an increase in Na^+ permeability, and hyperpolarizing responses due to an increase in either Cl^- or K^+ permeabilities. Hyperpolarizing K^+ aspartate responses have not as yet been found. As is seen in Table 1, the times to peak of the Na^+ and Cl^- conductance changes are much more rapid than the K^+ conductance change, and the values for the same ionic conductance to different putative transmitters are very similar. These values are comparable to those reported for serotonin (*15*) and dopamine (*16*). In all cases the aspartate and glutamate responses are slightly slower than those to ACh, but this may reflect the fact that the aspartate and glutamate receptors are located in the neuropil, whereas the ACh receptors are on both soma and neuropil. Similar differences in the times to peak for soma versus neuropil responses have been reported (*17*).

Specific aspartate receptors have not previously been found in either vertebrate or invertebrate preparations, although suggestions have been made on the basis of steric models about aspartate-preferring or glutamate-preferring receptors (*8, 18*). Aspartate has been reported to be a more potent excitant than glutamate on Renshaw cells and some brainstem neurons (*19*). Aspartate has also been termed a modulator or "cotransmitter" at the lobster excitatory neuromuscular membrane. In this preparation the binding of aspartate enhances the binding of glutamate and the subsequent depolarization of the membrane potential (*3*). No similar interactions be-

tween aspartate and glutamate have been seen in *Aplysia*.

In *Aplysia*, the receptors activated by iontophoretic application of serotonin and acetylcholine appear to be identical to those existing at serotonergic and cholinergic synapses, respectively (*14, 15*). Our results suggest that aspartate may also function as a neurotransmitter since there are specific receptors for aspartate which can selectively change the membrane permeability of some neurons to Na^+ or Cl^- (or both).

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Hydrogen Cyanide Formation over Automotive Catalytic Converters

In a report in *Science* (*1*) Voorhoeve *et al.* discussed laboratory experiments in which substantial amounts of HCN were generated when reactor gas was passed over Pt oxidation catalysts. This topic is significant since many late-model automobiles use Pt alloy oxidation catalysts for the control of CO and unburned hydrocarbon compounds. In addition, the authors point out (*1*): "Future application of catalytic converters for the reduction of NO_x (oxides of nitrogen) is a strong possibility, especially in the remainder of this decade. . . . In some proposed devices for NO_x reduction, a Pt catalyst is used in a reducing exhaust mixture as an oxygen scavenger upstream from the proper NO_x catalyst."

Gould, Inc., manufactures an NO_x reduction catalyst which is similar to the preceding description (*2*). The system is designed to operate at carburetion slightly rich of stoichiometry to provide a net reducing atmosphere. One element of the catalyst system is a Pt alloy, oxygen-removal catalyst situated upstream of the base metal NO_x catalyst bed.

Several comments on the relevance of the data of Voorhoeve *et al.* to an actual automotive exhaust environment can be made. Their reactor feed gas contained 0.5 percent H_2, 5 percent CO, 0.1 to 0.5 percent NO, 0 to 0.006 percent SO_2, and 0 to 3.5 percent H_2O. Although this gas is an approximation to automotive exhaust with very rich carburetion, the correlation is not good. Vehicle exhaust typically contains additional components: 10 percent H_2O, 12 percent CO_2, and 0 to 1 percent O_2. Laboratory data obtained from gas containing these additional components have shown that H_2O, CO_2, and O_2 in the exhaust gas can significantly affect the amount of HCN formed over a Pt catalyst. Moreover, the Pt catalyst tested by Voorhoeve *et al.* promoted complete NO conversion with high selectivity toward NH_3 formation. This particular situation does not occur in a catalyst system where the oxygen-removal catalyst has been treated to eliminate NH_3 formation. The decreased selectivity toward NH_3 formation is observed to influence HCN formation.

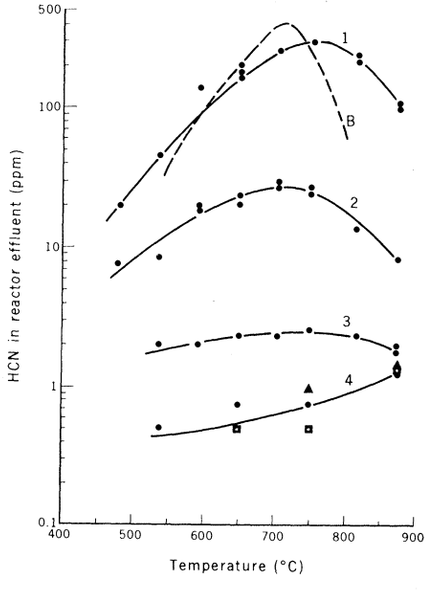

Fig. 1. Formation of HCN over Pt alloy and base metal catalysts: curve 1, PTX in extreme (to maximize HCN formation) exhaust gas containing no H_2O; curve B, data of Voorhoeve *et al.* (*1*) (sponge Pt-II) under conditions similar to those of curve 1; curve 2, PTX in extreme exhaust gas containing 5 percent H_2O; curve 3, PTX in extreme exhaust gas containing 10 percent H_2O; and curve 4, untreated PTX (•), treated PTX (▲), and GEM 68 (□) in typical synthetic exhaust gas containing 10 percent H_2O.

We have carried out simulated exhaust gas and actual vehicle testing to check for HCN formation over the NO_x reduction catalyst system. In laboratory testing we used bottled gas with N_2 as the carrier gas. The exhaust gas delivery rate equaled a space velocity of 100,000 $hour^{-1}$. We measured the HCN by bubbling a measured volume of gas through a 0.1*M* KOH solution; the solution was titrated with 0.1*M* $AgNO_3$ with the use of a KI indicator. This technique was insensitive to feed gas moisture and detected HCN in exhaust gas concentrations as low as 0.5 part per million (ppm) with an accuracy of ± 5 percent of the measured value.

The oxygen-removal catalyst used with the NO_x catalyst was an Engelhard PTX-IIB monolith. In some of the experiments we used a PTX catalyst to which a thermochemical treatment had been applied that was effective in suppressing NH_3 formation. The actual NO_x reduction catalyst was the Gould GEM 68 formulation.

The laboratory data are shown in Fig. 1. Curve 1 was measured under the conditions outlined by Voorhoeve *et al.* to maximize HCN formation, that is, 5 percent CO, 0.3 percent NO, and 0.5 percent H_2. The superimposed dashed curve (curve B) represents the data of Voorhoeve *et al.* (*1*) measured under the same conditions except that the curve 1 space velocity was 100,000 $hour^{-1}$ and the curve B space velocity was 14,000 $hour^{-1}$. When 5 percent H_2O (curve 2) is added to the feed gas, the maximum amount of HCN formed decreases from 300 to 30 ppm. When H_2O is adjusted to the 10