the order of 10^{-8}, equivalent to 0.1 ppm of dry weight in the medium. The amount of vanadium required by the rat is also quite close to that calculated as the daily vanadium intake for man. A rat weighing 75 g needs 1 to 2 μg of vanadium per day (25 μg per 100 g of diet) for an optimal growth response, while a human with an average weight of 75 kg has been estimated to consume 2 mg of vanadium daily. This calculation is based on dietary surveys and "a well-balanced diet." The total amount in man's body is estimated to be 17 to 43 mg (*4*).

The chemistry of the natural occurring derivatives of vanadium is unknown. Much of the vanadium must be present in low molecular form since it tends to occur in fats and oils. The properties of vanadium make it likely that its biological function is related to oxidation-reduction mechanisms. Effects of vanadium have been observed in many systems. Notably, it catalyzes the in vitro oxidation of phospholipids by mammalian liver protein fractions (*28*). It is highly active as a catalyst in the nonenzymatic oxidation of catecholamines, dihydroxyphenylalanine, and 5-hydroxyindoles (*29*). At high dose levels it inhibits cholesterol synthesis (*30*) and lowers the amounts of phospholipid and cholesterol in the blood (*31*). It has also been reported to inhibit the development of caries through stimulation of the mineralization of teeth (*32*). Whether any of these phenomena are related to the normal function of vanadium in intermediary metabolism remains to be established. In a review on vanadium in man (*4*), Schroeder *et al.* stated in 1963: "No other trace metal has so long had so many supposed biological activities without having been proven to be essential." The above data show that vanadium is indeed essential for the growing rat.

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Social Behavior of Monkeys Selectively Depleted of Monoamines

Abstract. *Initiated social interactions of* Macaca speciosa *are decreased during the period of treatment with alpha-methyl-*p*-tyrosine, an inhibitor of catecholamine synthesis. The treated animals maintained stable body weights and appeared to be healthy. Similar depletion of indoleamines with* p*-chlorophenylalanine does not change these same observed behaviors in spite of weight loss, hair loss, ataxia, and debilitation in some of the animals.*

The monoamines serotonin, dopamine, and norepinephrine have been implicated in such activities as the regulation of appetite, temperature, sleep, alertness, motor functions, and mood (*1*). In addition, complex disturbances in behavior have been related to an excess or deficiency of one or more of these substances, as suggested, for example, by the recent successful treatment of Parkinson's disease by the dopamine precursor dihydroxyphenylalanine (*2*). Selective experimental depletion of the indoleamines or the catecholamines has only recently allowed study of specific monoamine functions with the use of inhibitors of enzymes required for synthesis. Parachlorophenylalanine (PCPA) effectively decreases synthesis of serotonin (*3*), and alpha-methyl-*p*-tyrosine (AMPT) specifically decreases both dopamine and norepinephrine by inhib-

iting the rate-limiting enzymes in the body and the brain (*4, 5*).

Previous studies with these enzyme inhibitors have shown a complex role for the monoamines in the regulation of sleep (*6*). Weight loss and death have also been reported with both compounds in a variety of species (*7, 8*). Parachlorophenylalanine causes bizarre behavior in rats and cats, including increased aggression, increased sexual mounting, and evidence of perceptual disorientation 4 to 5 days after treatment is started (*8, 9*). The effects of PCPA on active and passive avoidance responses in rats have been studied with differing results (*10*). Alpha-methyl-*p*-tyrosine produces "sedation" and decreased activity in a variety of species (*5, 11*). Decreased performance of rotorod walking and conditioned avoidance responses have also been described in several species with AMPT administration (*12*). The effects of both drugs in humans have been studied in low doses, or in disease states which might have prevented adequate central nervous system depletions or made their interpretation difficult (*4, 13, 14*).

We have studied the differing effects of selective monoamine depletion on the social interactions of nonhuman primates. These subjects are naturally social animals whose survival, like man's, depends on complex postural, facial, and vocal communications, many of which are interpretable by human observers. The phylogenetic position of these primates, in addition, might allow inferences about mood and feeling states not apparent in lower animals.

Baseline activities of four groups of five *Macaca speciosa* of mixed sexes housed in group cages were observed by different observers for 1 hour daily for 3 weeks by using ethological behavioral measurements we described previously (*15*). These included aggressive and submissive gestures and behavior, grooming, social-sexual presentations, mounting, and copulation. Four animals, two animals in each group, were treated orally or by nasogastric tube for a 14-day period with doses of PCPA or AMPT estimated to reduce serotonin or norepinephrine levels to 30 percent of normal (*16*). One animal, previously treated with AMPT, was given PCPA after an interval of 4 months to test the effects of both compounds on the behavior of a single animal. Half of the observers in each experiment were "blind" as to which animals were treated and with respect to the behavioral hypotheses under investigation. Analyses of variance, regression analyses, and *t*-tests of drug effects were indistinguishable for the animals treated with each compound, regardless of the knowledge or naiveté of the observers. We have consequently analyzed and reported them together.

Behaviorally, the AMPT-treated animals showed a consistently reduced level of initiated social interactions compared with their own baseline ($P < .0005$) and with the controls ($P < .05$) during the first 2-week period (*17*).

Table 1. Mean initiated social activity levels of treated and control animals per observation hour. Probabilities (*P*) of differences between means of treated animals and controls were calculated by Student's *t*-test. A paired *t*-test for the difference between the means was used to compare treatment and posttreatment with pretreatment periods (*18*). The *P* values for treated versus control animals refer to controls at the same time stage of the experiment as the treated animals in question. The *P* values for controls "versus pre-Rx" refer to controls at the various time stages of the experiment versus the same controls in the preliminary 3-week period. Abbreviations: S.E., standard error of the mean; pre-Rx, pretreatment; NS, not significant.

Pretreatment (3 weeks)			Treatment								Posttreatment (3 weeks)			
			First 2 weeks				Second 2 weeks							
Mean	S.E.	Versus controls $2P =$	Mean	S.E.	Versus pre-Rx $P =$	Versus controls $P =$	Mean	S.E.	Versus pre-Rx $P =$	Versus controls $P =$	Mean	S.E.	Versus pre-Rx $P =$	Versus controls $2P =$
AMPT-treated animals ($N = 4$)														
17.4	1.5	NS	7.9	1.3	0.0005	0.05	7.6	1.37	0.0005	0.025	15.7	2.1	NS	NS
Control animals ($N = 6$)														
18.5	4.0		17.8	3.8	NS		24.9	5.8	NS		24.1	6.7	NS	
PCPA-treated animals ($N = 4$)														
20.4	4.2	NS	27.5	9.3	NS	NS	*				33.8	5.1	NS	NS
Control animals ($N = 6$)														
20.6	4.1		28.5	10.5	NS						33.4	8.2	NS	

* Two animals were treated for an additional period of 2 weeks. They became increasingly debilitated at the same time that urinary 5-HIAA excretion decreased to 13 percent of the pretreatment levels. Their initiated social interactions increased insignificantly in spite of continued treatment with PCPA and their poor physical condition.

Table 2. Mean urinary excretion of 5-HIAA and MHPG, in micrograms per 24 hours, by AMPT- and PCPA-treated animals. Probabilities (*P*) of differences between the means were calculated by a Welch's *t*-test (*23*).

Pretreatment (3 weeks)		Treatment								Posttreatment (3 weeks)			
		First 2 weeks				Second 2 weeks							
Mean	S.E.	Mean	S.E.	Versus pre-Rx $P =$	Percent of pre-Rx	Mean	S.E.	Versus pre-Rx $P =$	Percent of pre-Rx	Mean	S.E.	Versus pre-Rx $2P =$	Percent of pre-Rx
AMPT-treated animals: MHPG excretion													
324	46.3	96	2.99	$< .025$	29	92	10	$< .005$	28.4	607	92	$< .02$	187
PCPA-treated animals: 5-HIAA excretion													
968.7	123.6	291.5	59.4	$< .0025$	30.1					572.6	135.6	$< .05$	59
PCPA-treated animals: MHPG excretion													
293.5	26.7	231.8	47.3	.50	78.9					362.2	43.0	$< .20$	123

This level was further reduced by continuing treatment at the same dosage level for an additional 2 weeks (see Table 1). The animals continued to tolerate normal interactions initiated by control animals and did not retreat spatially as has been observed with other drugs such as phencyclidine and phenobarbital (*18*). They remained in apparent physical health as determined by stable body weights, normal urinalyses, normal complete blood counts, and normal blood urea nitrogens. No tremor, ataxia, or hair loss was seen, although their movements sometimes appeared to be retarded. They assumed energy-conserving postures with their heads down and eyes sometimes closed. They appeared to notice, but to be uninterested in, surrounding cage activities. Two of four animals irreversibly lost social rank during treatment. Of the two whose rank did not change, one was the dominant male and the other, a female, was already lowest ranking.

The PCPA-treated animals, on the other hand, all maintained unchanged levels of total initiated and passive social activity (see Table 1). Two animals increased total initiated social activity and two animals decreased social activity insignificantly during treatment. After treatment two animals insignificantly increased social activity levels compared with the pretreatment period, and two animals decreased social activity from that level. Physical appearance and general condition varied from no apparent change in two animals to decrease eating, decreased drinking, and weight loss in two animals, both of whom appeared to be near death and had to be maintained with nasogastric intubations of fluids. Gaunt masklike facies was seen in one animal, and ataxia, hair loss, and apparent paresthesias were noted in another (*19*). All of the animals remained alert and interested in cage activities, and no abnormal postures were seen. Aggressive behaviors, such as threats or attacks, and sexual mounting, presentation, and copulation were not increased (*20*). Two animals were treated for an additional 2-week period. Although these animals appeared to become more ill and debilitated, their total initiated behaviors increased insignificantly. One animal who was most debilitated during this treatment lost two places in social rank. She recovered one place 1 week after treatment was stopped, and the other place was recovered after 12 weeks. Because of the apparently severe toxic effects in the first two PCPA-treated animals, the remaining animals' treatment was discontinued after the second week.

Almost identical decreases in urine levels of two major metabolites of norepinephrine and serotonin were achieved during the 2-week treatment periods with each inhibitor as measured in 24-hour collections from temporarily isolated monkeys. 3-Methoxy-4-hydroxyphenylethylene glycol (MHPG) was determined by the method of Dekirmenjian and Mass (*21*), and 5-hydroxyindole acetic acid (5-HIAA) was determined by the method of Korf and Valkenburg-Sikkema (*22*). Those animals treated with norepinephrine depletor AMPT showed a significant decrease of MHPG to 29 percent of the pretreatment level ($P < .025$) (*23*) (see Table 2). Similarly, 5-HIAA excretions during PCPA administration decreased to 30 percent of the pretreatment level ($P < .0025$). Determinations of MHPG on the same samples also decreased to 79 percent (P not significant), suggestive of previous reports of the lack of complete specificity of PCPA (*24*).

The behavioral consequences of these experiments are of interest because such clear changes in social behavior and appearance occurred with catecholamine depletion. Such changes were not seen when indoleamines were similarly depleted, in spite of the extreme debilitation of some of the serotonin-depleted animals. The behavioral effects of AMPT are similar to those seen in monkeys treated with reserpine, which depletes all the biogenic monoamines (*25*), or to those seen in monkeys separated from familiar environments or significant "others" (*26*). The changes in posture, facial expression, motor activity, and social initiative might also be compared to the depressive states seen in man. These changes may be important, we believe, because the biochemical intervention with AMPT is specific to dopamine and norepinephrine without affecting serotonin or other brain substances, indicating that a "depressive syndrome" may be produced consistently by catecholamine depletion alone.

In an identical behavioral and social model, a corresponding and similar decrease of serotonin synthesis produced a variable and quite different picture in which physical illness in some of the animals was the most apparent change. In spite of this, all of these animals continued to interact at the usual levels and to show interest and involvement in activities around them as determined by these same measurements of social behavior. Few comparisons could be made to social effects seen previously with PCPA in lower mammals or to depression as seen in man.

If monkeys are indeed capable of varying levels of "affect," this study would seem to support a catecholamine, as opposed to an indoleamine, hypothesis for "depression" (*27*) in this species. It suggests also a possible behavioral and biochemical model for further study which might be relevant to humans.

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Operant Conditioning of Specific Patterns of Neural and Muscular Activity

Abstract. *In awake monkeys we recorded activity of single "motor" cortex cells, four contralateral arm muscles, and elbow position, while operantly reinforcing several patterns of motor activity. With the monkey's arm held semiprone in a cast hinged at the elbow, we reinforced active elbow movements and tested cell responses to passive elbow movements. With the cast immobilized we reinforced isometric contraction of each of the four muscles in isolation, and bursts of cortical cell activity with and without simultaneous suppression of muscle activity. Correlations between a precentral cell and specific arm muscles consistently appeared under several behavioral conditions, but could be dissociated by reinforcing cell activity and muscle suppression.*

In investigating the possible role of precentral "motor" cortex cells in generating voluntary movements, previous experimenters trained monkeys to perform specific motor responses by making operant reinforcement contingent on the position and force trajectories of the responding limb (*1, 2*). Such response patterns involved coordinated activity of many muscles of the responding limb (*2, 3*) and therefore were not designed to resolve the question of which specific muscles a given cortical cell may influence. To determine the degree to which precentral cell activity may be correlated with specific limb muscles and to test the stability of such correlations during different behaviors, we recorded the activity of single precentral cells and four major arm muscles (a flexor and extensor of wrist and elbow) while reinforcing specific patterns of activity in these elements.

Experiments were performed with a fluid-deprived monkey (*Macaca mulatta*) seated in a restraint chair with his head immobilized and a juice-dispensing tube in his mouth. The monkey's arm could be held semiprone in a molded cast pivoted at the elbow, allowing measurable flexion and extension of the elbow but no gross movement of the wrist. The cast could also be locked in place (elbow at 90°, wrist at 180°), rendering all muscle contractions isometric (*4*). Electromyographic (EMG) activity of major flexors and extensors of the wrist (flexor carpi radialis and extensor carpi radialis) and elbow (biceps and triceps) was recorded through pairs of braided stainless steel wires permanently implanted in the belly of each muscle and led subcutaneously to a connector implanted on the skull. Activity of single precentral cells in contralateral cortex was recorded with tungsten microelectrodes. For about a month prior to the data-recording sessions, the monkey was trained in several behavior performances: (i) He was reinforced with fruit juice for sitting quietly while we tested the cells' responses to passive movement of the arm and cutaneous stimulation. (ii) With his arm in the position monitor he was reinforced for active flexion and extension of the elbow. (iii) With the position monitor locked in place, reinforcement was made contingent upon isometric contraction of any one of the muscles with simultaneous suppression of activity in the other three.

Specific patterns of cell and muscle activity were monitored and reinforced with an electronic "activity integrator" which continuously integrated a weighted sum of voltages proportional to cortical cell and muscle activity, and delivered a reinforcement when the resultant voltage exceeded a preset threshold (Fig. 1). The activity integrator had several input channels which accepted either voltage pulses triggered from the cell's action potentials or rectified EMG activity from specific muscles. A summing network produced a weighted sum

$$V(t) = \sum_i a_i v_i(t)$$

of these input voltages; the algebraic sign and magnitude of each weighting factor a_i were determined by the experimenter through a polarity switch and gain control for each channel. This summed voltage was temporally integrated with a parallel resistor-capacitor network with a time constant of 50 to 100 msec to generate the "integrator voltage." When this integrator voltage reached a preset threshold level V_T the feeder discharged and the integrator voltage was briefly reset to zero.

To illustrate a typical application, consider reinforcing the activity of a specific muscle, say biceps, in isolation. When cortical unit activity did not enter into the reinforcement contingency, its contribution to the integrator voltage was switched off ($a_5 = 0$). The polarity switches that were on the muscle channels were set such that activity in the biceps drove the integrator voltage toward threshold ($a_3 > 0$), while activity in the other three muscles drove the voltage away from threshold ($\alpha_i < 0$; $i = 1,2,4$). When reinforcement became available, the monkey typically began to emit bursts of EMG activity in several arm muscles every few seconds. The gain controls (a_i) were set such that approximately half of these burst responses were reinforced. After sev-