chromosome 21–linked AVP gene product.

In light of the above finding, it should be noted that Down's syndrome patients show an increased incidence of leukemia of all types (*14*). Furthermore, in one early study by Shein and Enders primary human cells transformed by SV40 virus showed a high frequency of chromosome rearrangement and monosomy involving the G group chromosomes (*15*). It is possibly significant that the gene that regulates the expression of the antiviral state can be assigned to chromosome 21, and that SV40-induced cell transformation and the incidence of leukemia also involve the same chromosome group.

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Morphine and Ethanol: Selective Depletion of Regional Brain Calcium

Abstract. *Administration of morphine or ethanol to rats produces a decrease in regional brain calcium in vivo. This effect is selectively antagonized by the stereospecific narcotic antagonist naloxone. Reserpine and the dopamine-acetaldehyde conjugate salsolinol also produce a depletion of regional brain calcium, but only the salsolinol depletion is antagonized by naloxone. Experiments with naloxone provide evidence for two calcium-sensitive pools in the central nervous system.*

Calcium ions play an important role in the release of neurotransmitters and in neuronal excitability (*1, 2*). Moreover, calcium plays a key role in mechanisms of excitation–muscle contraction coupling and excitation–neurotransmitter secretion coupling (*3*). Calcium has been suggested as the primary transducer of many hormone receptor interactions (*4*) and as a coupling agent in certain central nervous systems (CNS) (*5*). However, despite the vast amount of research on calcium and neuronal systems in vitro, very little is known about the effects of various drugs, particularly psychoactive drugs, on the in vivo calcium concentrations in the CNS. It has been demonstrated, however, that calcium antagonizes morphine analgesia (*6*) and the development of tolerance to morphine (*7*), and the administration of morphine to mice produces a slight but significant decrease in whole brain calcium (*8*). In addition, morphine stimulates phospholipid metabolism and inhibits transport of calcium facilitated by phospholipids and gangliosides (*9*). Similarly, the effects of ethanol on brain calcium in vivo have received little attention, although it has been reported that both morphine and ethanol antagonize the release of acetylcholine from whole brain and cortex slices (*10*). To further evaluate the role of calcium in psychoactive drug action, we have studied the in vivo effects of morphine and ethanol on regional brain calcium.

Brain calcium concentrations were determined by atomic absorption spectroscopy with a Perkin-Elmer model 303 spectrophotometer. Male rats (Sprague-Dawley, 150 to 200 g) were decapitated after appropriate drug treatment; the brains were immediately removed and rinsed in five washes of ice-cold isotonic saline to remove as much blood as possible. Use of ice-cold saline also facilitated the regional dissection of tissue. After the tissue was blotted dry, regional brain areas were dissected according to the outline of Glowinski and Iverson (*11*). Portions of tissue (25 to 50 mg) were transferred to weighed disposable test tubes, 200 μl of concentrated HNO_3 (analytical grade) was added to each tube, and the contents were evaporated to a dry white ash over a hot plate. The residue was allowed to cool for 15 minutes and resuspended in a solution of 0.5 ml of 0.1*N* HCl plus 4.5 ml of 1 percent lanthanum (as the oxide) in 0.01*N* HCl.

Four regional areas were examined: corpus striatum, cortex, hippocampus, and hypothalamus. As seen from Table 1, administration of morphine sulfate (25 mg per kilogram of body weight) produced a significant decrease in regional brain calcum. This decrease was dependent on dose over the range 5 to 50 mg/kg, linear over the analgesic range 5 to 25 mg/kg, and saturable at doses greater than 50 mg/kg. To determine whether the effect would be classified as a specific narcotic action, we attempted to block the agonist action with a specific antagonist. Naloxone hydrochloride has essentially no agonist effects except at high doses (50 mg/kg) and is highly stereospecific in antagonist activity (*12*). Naloxone inhibited the morphine-induced calcium depletion and showed no significant calcium-depleting effects when administered alone. The specificity of the calcium-depleting effect of morphine was further tested by giving reserpine, which has been reported to lower brain calcium levels (*13*). Although reserpine (5 mg/kg) caused an average regional depletion of 38 percent, the reserpine-

Table 1. Effects of psychoactive agents on regional brain calcium. Brain regions have been designated as follows: HYP, hypothalamus; HIP, hippocampus; CS, corpus striatum; and CTX, cortex. Drugs were injected intraperitoneally and animals were decapitated. The doses and times between injection and decapitation are: morphine sulfate, 25 mg/kg, 30 minutes; naloxone hydrochloride, 1 mg/kg, 15 minutes (naloxone controls); reserpine (Serpasil), 5 mg/kg, 120 minutes; pentobarbital (Abbott), 40 mg/kg, 30 minutes; and ethanol, 1.5 mg/kg administered as a solution of 50 gram percent ethanol in saline, 120 minutes. Salsolinol (50 mg/kg, 30 minutes) was administered 30 minutes after pyrogallol (see text). In antagonism experiments naloxone was given 15 minutes before the second drug, and naloxone controls were kept alive for the duration of the second drug's action. Controls receiving pyrogallol + naloxone showed no change in brain calcium. Each experiment consisted of three controls and three tests, and was replicated at least three times; 9 to 12 animals were used in each experiment. Results are reported as micrograms of calcium per gram of wet tissue. Each value is the mean ± standard error of the mean. Decreases were significant ($P = .01$) by Student's t-test.

Drug	Calcium content of brain region (μg/g)			
	HYP	HIP	CS	CTX
Control (0.9 percent NaCl)	58.2 ± 1.8	59.8 ± 1.8	56.8 ± 1.7	54.5 ± 1.6
Morphine sulfate	39.8 ± 2.0	37.4 ± 1.7	35.5 ± 2.4	36.8 ± 1.0
Naloxone hydrochloride	53.0 ± 1.0	53.2 ± 1.0	52.6 ± 1.1	52.7 ± 1.7
Reserpine (Serpasil)	38.5 ± 1.4	34.4 ± 3.5	33.1 ± 2.6	34.3 ± 1.7
Morphine + naloxone	52.3 ± 1.2	53.1 ± 1.8	51.1 ± 0.9	52.3 ± 0.9
Reserpine + naloxone	37.0 ± 3.1	35.2 ± 2.8	37.1 ± 1.1	34.8 ± 1.6
Ethanol	33.4 ± 3.3	33.4 ± 2.6	35.8 ± 1.7	34.8 ± 2.6
Salsolinol	35.2 ± 1.8	33.1 ± 2.1	31.8 ± 3.3	32.8 ± 2.1
Ethanol + naloxone	53.8 ± 2.4	53.0 ± 1.9	53.3 ± 1.2	53.2 ± 1.0
Salsolinol + naloxone	52.4 ± 2.1	52.8 ± 1.8	52.1 ± 2.4	50.4 ± 1.7
Pentobarbital	58.2 ± 2.2	52.0 ± 1.7	52.3 ± 2.8	52.4 ± 2.5

induced depletion was not altered by naloxone.

It has been suggested that alteration in calcium levels may be related to general CNS depression (*14*). Since morphine caused depression as well as depletion of calcium, we chose another CNS depressant drug and examined its calcium-depleting effect. Pentobarbital was administered at a dose that caused loss of the righting reflex in less than 15 minutes (40 mg/kg); however, no reduction of regional brain calcium was detected.

To compare the effects of morphine and ethanol on regional brain calcium, we administered ethanol at a dose which did not measurably affect the animals' behavior. Ethanol (1.5 mg/kg) produced a decrease in regional calcium that was significantly antagonized by naloxone. Since morphine and ethanol caused a significant depletion of calcium which was antagonized by naloxone, while the reserpine-induced calcium depletion was unaffected by naloxone, we considered the possibility that the effect of morphine and ethanol may represent a common biochemical action. To further explore this effect, we tested salsolinol, a tetrahydroisoquinoline alkaloid, for its ability to deplete regional brain calcium. Salsolinol is an alkaloid formed by nonenzymatic condensation of dopamine with acetaldehyde. Compounds of this class act as false adrenergic transmitters and have been proposed as possible contributors to some of the pharmacological actions of ethanol (*15*). Salsolinol (50 mg/kg) produced a significant decrease in brain calcium which was antagonized by naloxone. In vitro observations have suggested that some tetrahydroisoquinoline alkaloids are substrates for catechol-*O*-methyl transferase, and that prior treatment with pyrogallol prevents their degradation (*16*). For this reason, animals receiving salsolinol were given pyrogallol (250 mg/kg) 30 minutes before salsolinol treatment or (controls) before decapitation.

The data in Table 1 indicate that ethanol and morphine produce a very significant degree of calcium depletion. This depletion is antagonized by naloxone. Salsolinol, when administered alone, also depletes tissue calcium and is antagonized by naloxone.

The mechanism of calcium depletion is not understood, but it appears that the depletion by morphine of membrane-bound calcium is prevented by the selective action of naloxone. A fundamental event in the excitation of the membrane is dissociation of calcium from negatively charged binding sites. Major candidates for these sites are the acidic lipids such as phosphatidylserine, phosphoinositides, and gangliosides, all of which have a high affinity for divalent cations (*2, 17*). Thus, any disruption of this Ca^{2+}-membrane interaction may contribute in part to the observed effects of morphine and ethanol on neuronal systems.

Knowing that calcium is necessary for optimum neurotransmitter activity and neuronal membrane function (*2, 17*), it is tempting to speculate that the metabolic effects and alteration of neurotransmitter activity seen after administration of morphine and ethanol may be based on the initial disturbance of optimal membrane calcium associations. As for the effects observed with salsolinol, other investigators have suggested that tetrahydroisoquinoline alkaloids may be formed during ethanol ingestion and may play a role in the development of tolerance to and physical dependence on alcohol (*18*). However, until there is more conclusive evidence that these alkaloids are formed in vivo after ethanol ingestion in mammalian systems, this idea must be regarded as highly speculative. Since neither ethanol nor salsolinol structurally resembles morphine, and there appears to be no available pathway for converting them to such a structure, it is difficult to see how they can act directly to deplete calcium. However, the naloxone antagonism appears to be specific and further experiments are needed to characterize this differentiating effect.

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More on Seasonal Variations in Goldfish Learning

Goldfish obtained in weekly shipments from Ozark Fisheries, Stoutland, Missouri, demonstrate a seasonal fluctuation in ability to learn a shock-avoidance shuttle box task (*1*). High acquisition and retention scores are seen in February, with lowest ones in July. Shashoua reported a similar distribution of scores in a "float training" experiment in which animals adapt to a polystyrene float sutured to the ventral surface (*2*). He also confirmed the seasonal variation in shock-avoidance shuttle box performance (*3*). Fjerdingstad (*4*), however, saw no seasonal variability in a shock-avoidance task with goldfish obtained from Ozark or from another source. He suggested that the fish used by us and by Shashoua may be weak during the hot summer months as a result of the stress of shipping and starvation in the laboratory, and stated that we use the fish 1 or 2 days after arrival.

Fjerdingstad's last point is an incorrect assumption; we keep the fish in group tanks for at least 2 weeks before setting them out in individual home tanks the day before a behavioral experiment. We do not feed them unless they are kept longer. Furthermore, while fish fare better under cooler conditions of shipment than warm ones, it is not likely that temperature during shipment is the key to the problem of seasonal variation in avoidance conditioning. We have been receiving goldfish weekly for 10 years. Since 1970 we have discontinued using rail shipment in favor of air freight, without noticeable alteration in the seasonal pattern. If we kept summer fish at 8°C for several weeks before training, the performance typical of winter fish was not restored. Winter fish maintained in the laboratory at 19°C, under constant light and with daily feeding, did not show a decline in performance with the change in season.

Fjerdingstad's failure to see seasonal variation is probably due to other factors. Acquisition scores for goldfish in shuttle boxes vary according to stimulus parameters and to the apparatus (*5*). In the case of goldfish, the height of water over the barrier is especially important. In our experiments trials are massed in a single session, while Fjerdingstad trains fish over a period of days. If less effort and skill is involved in Fjerdingstad's task than in ours, one might expect less difference between groups of fish in varying states of vigor.

We conclude that the seasonal variation in behavior is a result of changes in the goldfish we receive. The origin of the variation is uncertain. Ozark Fisheries ships us fish 6 to 7 cm in body length throughout the year. The goldfish breeds annually in early summer, so the average age of their fish population must increase and then decrease annually. Fish are raised in about 400 ponds that are separated by levees and drained periodically, and the new generation is usually shipped beginning in September, so fish 6 to 7 cm long are rarely more than 2 years or less than 5 months old. We propose that, beginning in May or June, an increasing number of the fish we receive are the slower-growing individuals of the previous year (*6*). Beginning in the fall, we obtain the most vigorous individuals of the new generation. The resulting selection as a function of season could account for behavioral differences.

It is also possible that annual variations in the physiological state of the individual contribute to the seasonal changes in avoidance conditioning. Annual changes in photoperiod and temperature may be important. There are many precedents in biology. If Otto Loewi had searched for slowing of the frog heart in the summer, he might not have succeeded in discovering acetylcholine, since the inhibitory response of the frog heart is weak in the summer (*7*). Ovarian function is correlated with goldfish learning, as Shashoua notes (*2*), but the relevance of this observation to behavior is not clear for several reasons: (i) a large portion of 7- to 8-g female fish used by Shashoua may not be sexually mature; (ii) we have seen 8- to 11-g gravid females, some of which spawned abortively during training, perform well in the shuttle box; and (iii) presumably half of the fish are males. A correlate of ovarian but not of testicular function would result in a bimodal distribution of performance in summer fish. The standard error of the mean for a mixed sex population should thus increase during the summer. Examination of published data suggests that this is not the case (*1*, *2*).

Whatever the explanations are for the seasonal variation, storing winter fish in the laboratory may be a solution to the practical problem. Winter fish can be inexpensively fed and maintained in a limited space, and they appear to be suitable experimental subjects in the following summer.

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