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Modulation of Cycloheximide-Resistant Memory by Sympathomimetic Agents

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GIBBS, M. F. *Modulation of cycloheximide-resistant memory by sympathomimetic agents* PHARMAC. BIOCHEM. BEHAV. 4(6) 703–707, 1976 – Amphetamine overcomes the amnesia caused by cycloheximide (CXM) provided it is administered closely following the learning trial In day-old chickens with one trial passive avoidance learning, there is a short-term, labile memory existing for 90 min following training under the influence of CXM. Amphetamine has been shown to keep the memory at precisely the level exhibited by the labile, cycloheximide-resistant memory trace at the time of injection Norepinephrine, methoxamine (an α adrenergic stimulant) and isoprenaline (a β adrenergic stimulant) each mimic the amphetamine effect in CXM-pretreated chickens. That the action of amphetamine could be due to its release of norepinephrine is supported by the finding that it could be blocked by both α adrenergic (piperoxane) and β adrenergic antagonists (propranolol). It has been suggested that this labile memory trace depends on the functioning of a sodium pump Norepinephrine may be modulating memory formation by an action on the sodium pump since in preliminary biochemical assays norepinephrine stimulated the sodium pump (Na^+/K^+ ATPase) activity in chicken forebrain total homogenate.

Norepinephrine Labile protein-independent memory α and β adrenergic stimulants Sodium pump α and β adrenergic receptor blockers

IN day old chickens memory for passive avoidance training has been shown to be a two stage process [9,21]. The first is a short-term, labile phase which declines to amnesic levels of retention in 90 min. The second process is a long-term memory storage which depends on the normal short-term labile phase and protein synthesis for its formation.

In a preceding paper [2] it has been shown that amphetamine counteracts the amnesic effect of CXM provided it is administered before the labile, protein-independent memory has declined. Under such conditions retention has been found to be directly related to the level of labile memory at the time of amphetamine administration (Fig. 4). Therefore, when retention is high shortly after learning, the administration of amphetamine at this time results in a very similar retention 3 hr later but when the retention has fallen to a low level because of the presence of CXM amphetamine is unable to improve the retention at 3 hr.

The central nervous properties of amphetamine have been linked to its action on several putative neurotransmitter systems. The most prominent action appears to be the release of norepinephrine and a number of actions have been demonstrated, all of which would be expected to increase the concentration of norepinephrine at synapses in the central nervous system. It releases norepinephrine [23] and inhibits monoamine oxidase activity [4]. Amphetamine also causes the release of dopamine and inhibits its reuptake [19]. Locomotor hyperactivity and aggressive behaviour have been attributed to this release of norepinephrine [19] whereas stereotyped behaviour has been attributed to the release of dopamine [12] Schrold and Squires [14] have suggested that amphetamine may have an effect on behaviour via a serotonergic (5HT) mechanism and it has also been reported that antihistamines will block some central nervous actions of d-amphetamines [12].

The present study was to determine whether the effect of amphetamine on memory could be linked to any of its postulated actions on transmitter release. It became evident that norepinephrine release was probably responsible for the effect of amphetamine on labile memory as norepinephrine and also the α noradrenergic stimulant, methoxamine and the β noradrenergic stimulant, isoprenaline could each reproduce the effects of amphetamine Two noradrenergic receptor antagonists – piperoxane and propranolol – were employed to determine if the amphetamine action was due to the release of norepinephrine However it was important to see whether the other postulated actions for amphetamine were involved and for that reason the drug haloperidol was used to block dopamine receptors, cyproheptadene was used to block 5HT receptors and mepyramine was used to block histamine receptors. The drugs chosen were standard pharmacological antagonists or agonsits of the transmitters involved [1].

Chickens have a reduced blood brain barrier for biogenic amines during the first month after hatching [18] EEG and behavioural responses to systemically administered

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biogenic amines are similar to those observed after direct application of amines into the brain of adult birds [16]. This reason made the subcutaneous administration of the drugs possible, this was desirable as the CXM was administered intracranially.

METHOD

Procedure

Environmental conditions and procedure are the same as reported previously [2], where a one trial passive avoidance learning task was employed with day old chickens Pecking of a normally attractive shiny metal bead was inhibited in a 10 sec presentation by coating the bead with an aversive chemical, methyl anthranilate. Retention tests were given at 3 or 24 when a non-coated bead was presented for 10 sec On these tests, retention was recorded as the percentage of chickens in groups of 20 or more which avoid the bead

Drugs and Injections

All drugs were made up in sterile NaCl (0.9% w/v). Cycloheximide (Actidione, Upjohn Co.) 20 μg/chicken, or saline was administered intracranially by freehand injection into each side of the forebrain in volumes of 10 μl per hemisphere using a Hamilton repeating dispenser syringe A stop on the syringe needle regulated the depth of injection to 3 mm. These injections were performed 5 min before the learning trial.

The other drugs were administered subcutaneously 10 min after the learning trial in volumes of 0 1 ml to chickens pretreated with CXM or saline. They were d-amphetamine sulphate (1.0 mg/kg), l-noradrenaline bitartrate (5.0–100 μg/kg); piperoxane (1.0 or 2 0 mg/kg); propranolol (1 0 or 2.0 mg/kg), haloperidol (1.0 or 2.0 mg/kg), mepyramine maleate (2.0 or 5.0 mg/kg); methoxamine HCl (50 μg/kg) and isoprenaline (50 μg/kg)

In experiments with the varius transmitter antagonists (piperoxane, propranolol, haloperidol, mepyramine, cyproheptadene) each was administered to CXM-pretreated chickens in the same injection as the amphetamine Control groups were injected with CXM and receptor blockers, saline and receptor blockers, or saline, receptor blockers and amphetamine. Similarly, for some experiments, piperoxane and propranolol were each combined with norepinephrine in a single administration In the other experiments, norepinephrine and noradrenergic agonists methoxamine and isoprenaline were each administered without amphetamine in CXM-pretreated chickens. In addition to the above injection time of 10 min after learning, subcutaneous norepinephrine was administered at times up to 120 min after learning to chickens pretreated with saline or CXM, enabling further comparison with earlier experiments with amphetamine.

RESULTS

With chickens pretreated with CXM, amphetamine had its maximum effect in preventing CXM-induced amnesia when administered close after the learning trial (Fig. 4). Amphetamine had no effect on memory retention in saline-pretreated chickens

Effect of Transmitter Antagonists on the Reversal by Amphetamine of CXM Amnesia

Piperoxane and propranolol (1.0 mg/kg) abolished the effect of amphetamine in CXM-pretreated chickens (Fig. 1A). Similar results were obtained with concentrations of 2.0 mg/kg. In the absence of amphetamine, neither of these drugs produced any change in memory retention in saline- or CXM-pretreated chickens.

The dopamine receptor antagonist – haloperidol (1.0 mg/kg), the histamine receptor antagonist – mepyramine (2.0 mg/kg) and the serotonin antagonist – cyproheptadene (1.0 mg/kg) injected with amphetamine did not alter the effect of amphetamine on CXM-induced amnesia (Fig. 2); similar results were obtained with the higher doses. None of these drugs had any action of their own without amphetamine in either CXM- or saline-pretreated chickens.

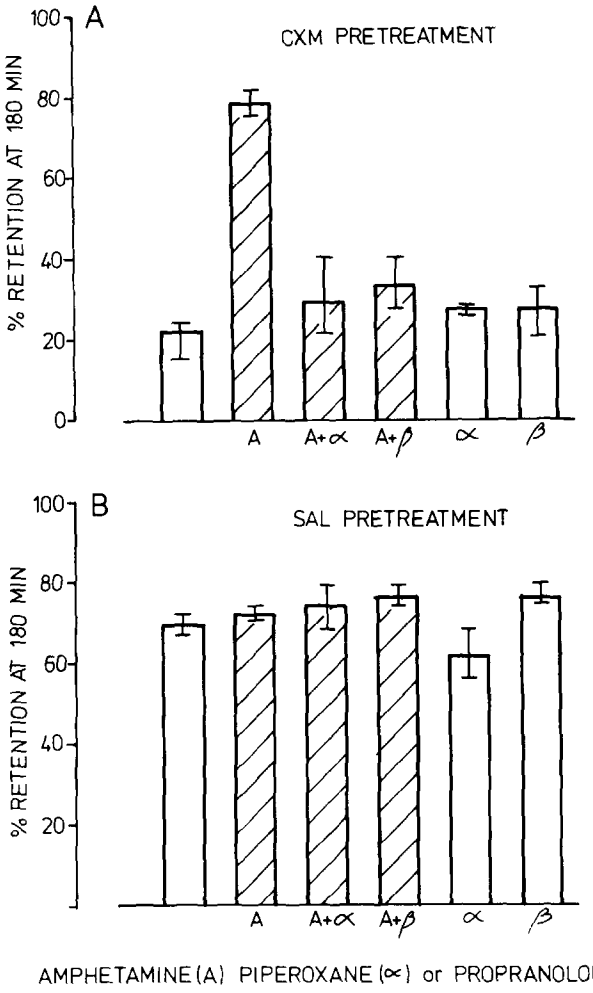

FIG. 1 Chickens were pretreated with CXM (A) or saline (B). 10 min after learning they received one subcutaneous injection of 1.0 mg/kg amphetamine and/or 1.0 mg/kg piperoxane (α blocker) or 1.0 mg/kg propranolol (β blocker). Each retention score represents the mean of 2–4 groups of 20 chickens and the bars represent the minimum and maximum percentage of the total number of groups tested under each condition. Using Rodger's [11] technique of planned contrast on proportions, CXM-pretreated chicks given amphetamine differed significantly in proportional retention from all other groups pretreated with CXM ($p<0.05$). The remaining groups were not significantly different from each other No significant differences were found between saline pretreated groups of chickens

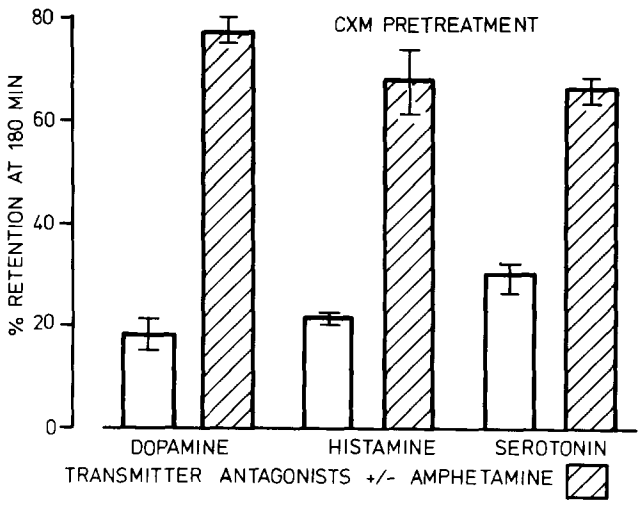

FIG. 2. Percentage retention following administration of the transmitter antagonists haloperidol (dopamine), mepyramine (histamine) or cyproheptadene (serotonin, 5HT), 1.0, 2.0, and 1.0 mg/kg respectively, to chicks treated with CXM or CXM and amphetamine (1 0 mg/kg). Amphetamine and the transmitter antagonists were administered 10 min after learning, while CXM was administered 5 min prior to learning. None of the transmitter antagonists prevented amphetamine overcoming the inhibition by CXM. Chicks pretreated with CXM and given haloperidol, mepyramine or cyproheptadene all differed significantly in proportional retention [11] from the respective groups given amphetamine as well ($p<0.05$).

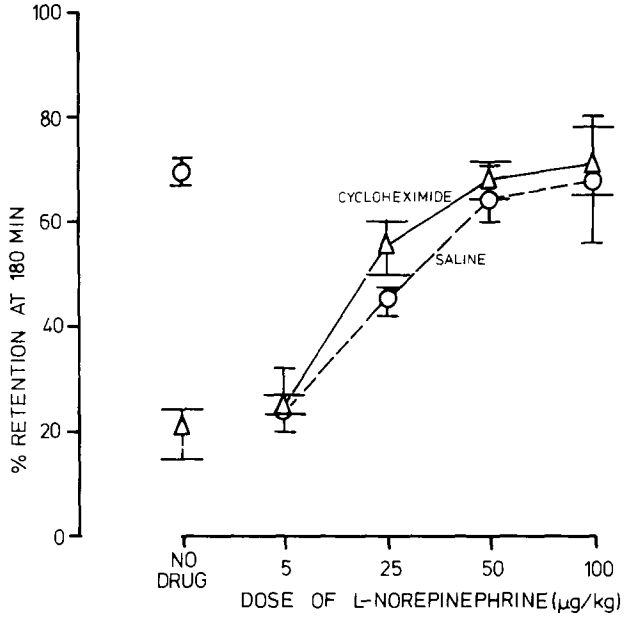

FIG. 3 Retention at 180 min in chickens injected with a range of concentration of norepinephrine 10 min after learning and pretreated with either CXM or saline. Fifty and 100 μg/kg norepinephrine did not affect those chicks treated with saline but antagonized the amnesic effect of CXM. The proportional retention of CXM-pretreated chicks receiving 25, 50, or 100 μg/kg norepinephrine differed significantly ($p<0$ 05) from chicks receiving no post-training treatment. Those receiving 5 μg/kg were not significantly different. Chicks pretreated with saline were only significantly different ($p<0$ 01) when they received 5 μg/kg. No other doses of norepinephrine were significantly different from saline-pretreated chicks receiving no post-training treatment.

These results with the transmitter antagonists suggest quite strongly that the pharmacological action of amphetamine responsible for its effect on labile memory is due to norepinephrine release because the α and β blockers block the effect of amphetamine in eliminating CXM-induced amnesia.

Dose-Response Curve for Norepinephrine

Four doses of norepinephrine, 5, 25, 50 or 100 μg/kg were given 10 min after learning. Retention was measured at 180 min in groups of chickens pretreated with CXM or saline (Fig. 3). With the high doses (50 and 100 μg), norepinephrine was able to counteract the amnesia induced by CXM but it had no effect on memory in chickens injected with saline.

The low dose of norepinephrine (5 μg/kg). inhibited memory formation in saline-pretreated chickens. Such a result is consistent with previously observed effects of a low dose of amphetamine (0.1 mg/kg) [2]. The inhibiting effect of a low dose of norepinephrine on memory retention in saline-pretreated chickens is curious and is currently under investigation.

Time of Norepinephrine Administration

Norepinephrine (50 and 100 μg/kg) mimicked amphetamine in overcoming CXM-induced amnesia. When norepinephrine (50 μg/kg) was administered subcutaneously 10 min after learning to chickens pretreated with CXM, the same retention was measured at 180 min as in the control saline-pretreated chickens (Fig. 4)

As the interval between the time of learning and the administration of norepinephrine became greater, norepinephrine became less effective in maintaining the memory. This effect was still evidenced in retention tests 24 hr after learning, so clearly norepinephrine is having an effect on memory and the results are not due to a change in performance. Its effectiveness was therefore dependent on the time of administration, as is that of amphetamine [2].

When the α and β receptor antagonists are administered with norepinephrine (50 μg/kg) they prevented norepinephrine reversal of CXM-induced amnesia (Fig. 5), a result similar to that found with amphetamine.

Noradrenergic Receptor Agonists

The noradrenergic agonists, methoxamine (50 μg/kg) and isoprenaline (50 μg/kg), which stimulate α and β adrenergic receptors respectively, were injected single and in combination into chickens pretreated with CXM (Fig. 6). When given 10 min after learning, retention testing at 180 min revealed reversal of CXM amnesia; i.e. these drugs mimicked the response to norepinephrine. A similar result was obtained when a higher dose (100 μg/kg) of either agonist was used. The effect with α and β agonists administered 10 min after learning was still apparent 24 hr later.

DISCUSSION

The present experiments indicate that the effect of

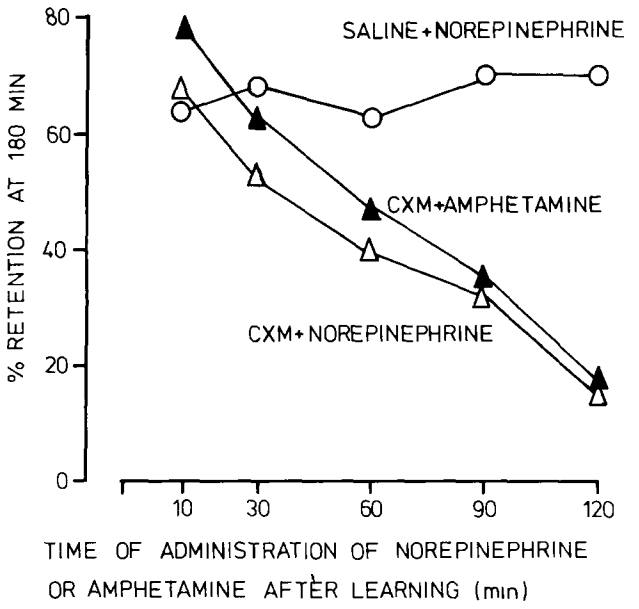

FIG 4 Retention at 180 min in chickens given intracranial CXM or saline before learning and injected with 50 μg/kg norepinephrine at intervals of 10–120 min after learning. Data from Gibbs [2] on CXM-pretreated chickens given amphetamine (1 0 mg/kg) at the same time intervals is included for comparison. Norepinephrine given 10 min after learning produced no significant difference ($p<0$ 05) between saline and CXM pretreated chicks From 30 min onwards there was a significant linear trend in the proportional retention of chicks receiving CXM-pretreatment plus norepinephrine Saline pretreated chicks showed no significant linear trend with norepinephrine treatment [11]

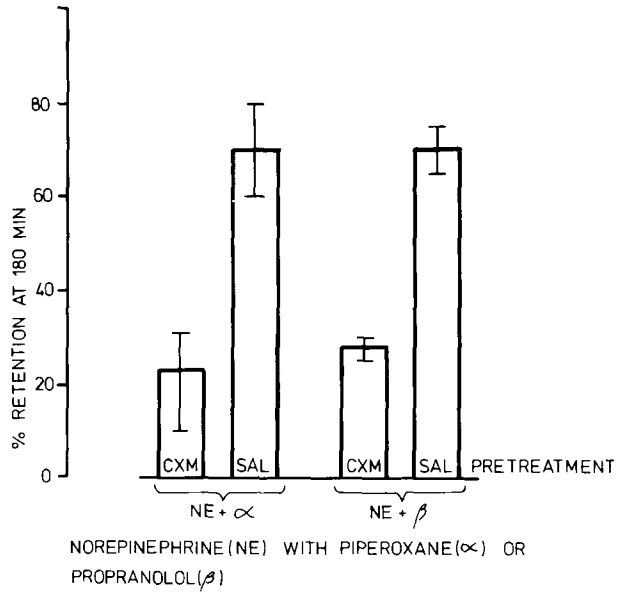

FIG. 5. Percentage of chickens avoiding on the retention trial 180 min after learning. Chickens pretreated with either CXM or saline were given a subcutaneous injection 10 min after learning of 50 μg/kg norepinephrine with either 1.0 mg/kg piperoxane (α antagonist) or 1 0 mg/kg propranolol (β antagonist) There was a significant difference ($p<0$ 05) between chicks receiving CXM plus norepinephrine and those receiving piperoxane or propranolol in addition. The differences between the saline pretreated groups were not significantly different from chicks receiving only saline plus norepinephrine.

amphetamine in reversing CXM-induced amnesia probably stems from its norepinephrine releasing property [23], and that both its α and β noradrenergic properties appear to be involved. Administration of either the α noradrenergic antagonist piperoxane or the β antagonist propranolol abolish the ability of amphetamine to reverse CXM-induced amnesia, whereas the other non adrenergic transmitter antagonists – haloperidol, mepyramine and cyproheptadene allow amphetamine to prevent CXM-induced amnesia. None of the drugs influence CXM amnesia without amphetamine, nor do they effect memory in control chickens pretreated with saline. Furthermore, norepinephrine (50 μg/kg) has a similar action to amphetamine and its effect is dependent on dose as well as on the time of administration after learning, this action can be antagonized by both α and β stimulants.

It is unusual to find an effect of norepinephrine that can be blocked equally well by both α and β receptor blockers In the peripheral nervous system α and β actions are usually separable and even in the central nervous system, different behavioural effects of epinephrine and norepinephrine on food intake, for example, can be shown to involve one or other of the two receptor types [8].

An alternative interpretation of the current results might be that norepinephrine is producing a direct behavioural effect that influences retention testing and is scored as memory. In rats, high doses of norepinephrine will produce stupor and abolition of motor activity, but when the dose is lowered, increases in locomotion and exploratory activity

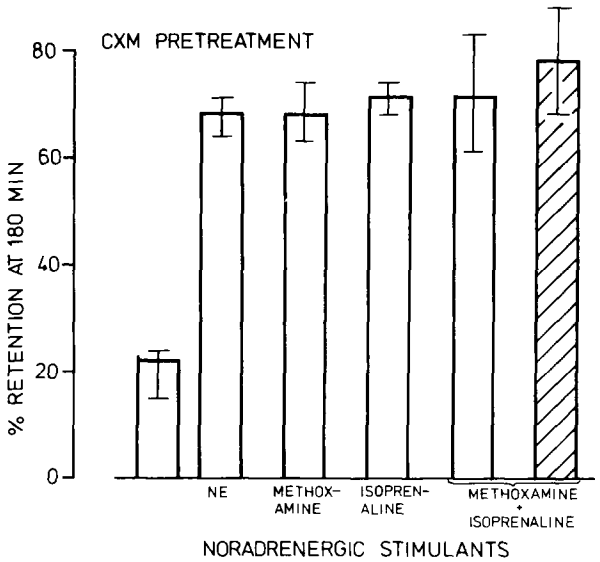

FIG. 6 Percentage of chicks avoiding at 180 min retention test when CXM-pretreated chicks were given different sympathomimetic drugs 10 min after learning Methoxamine (50 μg/kg) and/or isoprenaline (50 μg/kg) were compared with norepinephrine (50 μg/kg) post-training treatment, CXM-pretreatment only was also included for comparison. The hatched bar represents retention at 24 hr The proportional retention of chicks receiving CXM-pretreatment only differed significantly ($p<0.01$) from all other treatments, which did not differ significantly from each other using Rodger's planned contrasts [11]

have been reported [5,15]. In the present experiments where the norepinephrine dose is low, any increase in activity would decrease the apparent memory score, which is opposite to the results observed. In chickens, behavioural sleep, lowered temperature, lowered blood pressure and reduced oxygen consumption were reported for norepinephrine infused into the hypothalamic area, but there was no effect when infused into the cerebral hemisphere [10], and it is the latter area where CXM has been injected and shown to inhibit memory formation. These findings suggest it is unlikely that norepinephrine is falsely influencing retention testing.

There is evidence from experiments where different areas of the chick forebrain were injected with ouabain (Cherkin and Gibbs, unpublished data) that the neostriatal area is the most important for the inhibition of memory formation. Regional uptake of labelled norepinephrine is greatest in the paleostriatal and neostriatal regions of the chicken forebrain [17]. Thus the neostriatal region of the chicken forebrain may be involved in the inhibition of short-term memory by ouabain, the inhibition of long-term memory by CXM and possibly in the effect of norepinephrine in overcoming CXM amnesia

From the results presented in this paper one may speculate about a possible physiological basis for reinforcement of responses. Kety [7] has proposed that norepinephrine may be released as a result of arousal induced by significant or novel stimuli and that a heightened level of arousal may influence neuronal processes involved in memory. In terms of memory formation J. Z. Young [22] has suggested that an "address" is maintained If neuronal connections are modified by changes in protein synthesis as a result of learning, the individual synapses then become identifiable in terms of biochemical or physiological changes. Horridge [6] makes the point that "the address reinforcement acts upon is responsive and reveals itself because it has recently been active." This implies that the effects of reward and punishment are widespread and are not specific; it is only recently active circuits that need to be sensitive to reinforcement. The short-term, protein-independent, labile memory could possibly be a phase in memory storage where modulation, perhaps by reinforcement, could occur.

Previous experiments have indicated that the sodium pump is involved in the phase of short-term memory storage [3, 9, 21]. Other experiments have shown that the neuronal re-uptake of norepinephrine involves Na^+/K^+ ATP'ase [20]. Preliminary biochemical assays (Jeffrey and Gibbs, in preparation) have shown that norepinephrine, in a comparable concentration to the behavioural dose used in these experiments, doubles Na^+/K^+ ATP'ase activity in the chicken forebrain

If reinforcement is defined as keeping synapses identified or addressed for an increased period of time, then changes in norepinephrine levels, artificially or naturally induced, may do so by selective maintenance of the protein-independent or cycloheximide-resistant memory

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