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# Tryptophan and Tonic Immobility in Chickens: Effects of Dietary and Systemic Manipulations

Gordon G. Gallup, Jr., Larry B. Wallnau, James L. Boren, Gregg J. Gagliardi
State University of New York at Albany

Jack D. Maser
National Institute of Mental Health
Bethesda, Maryland

Patricia H. Edson
University of Arkansas at Monticello

Consistent with a serotonergic-midbrain raphe model of tonic immobility, four experiments designed to affect changes in serum tryptophan produced reliable effects on the duration of the response in chickens. Systemic injections of tryptophan, the dietary precursor to serotonin, led to a dose-dependent increase in immobility, with optimal effects being observed within 30 min after injection. Dietary depletion of endogenous tryptophan served to attenuate the duration of immobility, and a diet completely free of tryptophan, but supplemented with niacin, practically abolished the reaction. Dietary replacement served to reinstate the response. In a fifth experiment, tryptamine, an alternative metabolic by-product of tryptophan, was found to have no effect on immobility. The data are discussed in light of evidence showing serotonergic involvement in tonic immobility.

Tonic immobility, produced by a brief period of physical restraint, represents a state of motor inhibition and catatoniclike paralysis which may last from a few minutes to over several hours. Previous research (see Gallup, 1974) has implicated monoamines, especially changes in serotonin (5-HT), as having an important effect on the duration of tonic immobility in chickens. Maser, Gallup, and Hicks (1975) found that drugs thought to increase or simulate increases in synaptic concentrations of brain serotonin (e.g., LSD-25, BOL-148, pargyline, iproniazid) prolong the duration of tonic immobility. In a related study by Hicks, Maser, Gallup, and Edson (1975), birds receiving morphine sulfate showed exceptionally long durations of immobility, with those given relatively low doses exhibiting reactions lasting almost nine times longer than those of controls. As evidence for serotonergic mediation of the enhancement, pretreatment with parachlorophenylalanine, a compound thought to deplete brain serotonin, abolished the morphine effect.

Data from these studies parallel electrophysiological work by Aghajanian and his associates (e.g., Aghajanian, Foote, & Sheard, 1970; Aghajanian & Haigler, 1973), in which drugs that increase tonic immobility have been found to suppress electrical activity in mammalian midbrain raphe nuclei. The raphe nuclei are thought to be a major source of overall brain 5-HT, and a reduction in raphe firing has been taken to indicate excess serotonin in the synaptic cleft. An inverse relation appears to obtain between raphe electrical activity and tonic immobility. Drugs that increase raphe firing (e.g., amphetamine) decrease rather than increase tonic immobility (Boren & Gallup, 1976; Thompson, Piroch, & Hatton, Note 1). In further support of this parallel, Klemm (1971), on the basis of brain transection studies and electrical recording, postulated the existence of a tonic immobility control center somewhere in the medullary reticular formation, which is in sufficient proximity to many raphe nuclei to also implicate raphe neurons.

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Requests for reprints should be sent to G. G. Gallup, Jr., Department of Psychology, State University of New York at Albany, Albany, New York 12222.

The present series of experiments was designed to assess the effects of tryptophan, the essential dietary amino acid precursor to 5-HT, on tonic immobility in chickens. Fernstrom and Wurtman (1971a, 1971b) showed that alterations of serum tryptophan result in significantly changed concentrations of brain 5-HT in rats, and Aghajanian (1972) found suppressed raphe activity following administration of tryptophan. Therefore, if the duration of tonic immobility is related to serotonin levels, one would expect tryptophan injections to prolong the reaction by stimulating 5-HT synthesis in the brain, whereas depletion of endogenous tryptophan ought to diminish immobility.

## Experiment 1

Fernstrom and Wurtman (1971a) found that intraperitoneal injections of tryptophan in rats led to elevated brain 5-HT concentrations 1 hr after injection. With similar time and dose parameters, chickens in the present study were injected with tryptophan and tested for tonic immobility.

### *Method*

*Subjects.* The subjects were 40 straight-run, Production Red chickens (*Gallus gallus*) obtained from a local hatchery at 1 day of age. The birds were housed in commercial brooders and provided with continuous access to Purina Chick Chow (Growena) and water under conditions of 14 hr of artificial light per day.

*Apparatus and procedure.* Testing was conducted between 17 and 19 days of age; the three dosage levels selected (25, 50, and 125 mg/kg) were ones employed by Fernstrom and Wurtman (1971a). With an average body weight of 145 g, three groups of 10 birds received a .5-ml ip injection of 3.6, 7.3, or 18.1 mg of L-tryptophan methyl ester HCl dissolved in distilled water. The 10 remaining birds served as controls and received a .5-ml injection of distilled water. In an attempt to minimize the effects of handling on tonic immobility (Nash & Gallup, 1976), the birds were weighed after testing in order to calculate average drug doses, which were 27.9, 55.8, and 139.5 mg/kg of tryptophan for the three experimental groups, respectively.

Following the injection each bird was transported inside a cardboard box to a testing room. After 60 min had elapsed, the bird was taken from the box and tested for the duration of tonic immobility. Testing consisted of holding the bird on its right side with both hands and applying gentle pressure for a period of 15 sec. Immobility was measured with a stopwatch from the time of release until the bird showed a righting response and got back to its feet.[1]

### *Results*

Systemic injections of tryptophan, as predicted, served to potentiate tonic immobility, with larger doses in general leading to longer reactions (see Figure 1). Because of heterogeneity of variance, the data were subjected to a cubic transformation (Dunlap & Duffy, 1974), and an analysis of the transformed scores provided evidence of a significant difference between groups, $F(3, 36) = 5.36, p < .004$. Post hoc comparisons with Duncan's multiple range test revealed significant differences ($p < .05$) between the groups receiving tryptophan and the controls, but not between the two highest doses.

## Experiment 2

In rats, systemic injections of tryptophan produce increased concentrations of brain 5-HT which become maximal approximately 1 hr after administration

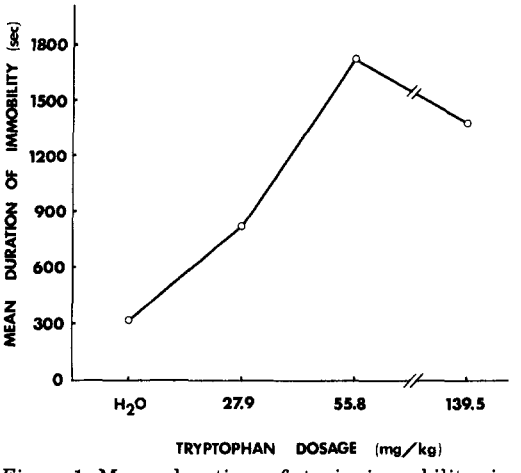


*Figure 1.* Mean duration of tonic immobility in chickens as a function of tryptophan dosage.

[1] Since there are daily rhythms in the concentration of plasma tryptophan, in all experiments reported in this article, testing, with order randomized across groups, was conducted between 1:00 and 5:00 p.m. when concentrations are typically low (Fernstrom, Madras, Munro, & Wurtman, 1974).

(Fernstrom & Wurtman, 1971a). Since avian physiology may be quite different from that of the rat, it seemed worthwhile to determine optimal injection–test intervals for tryptophan effects on tonic immobility in chickens as well as to provide a partial replication of the previous results.

### *Method*

The subjects were 26 Production Red chickens obtained and maintained similarly to those in the first experiment.

At approximately 3 wk of age the birds were randomly divided into three groups. Birds in each group were given an ip injection of 14.5 mg of L-tryptophan methyl ester HCl dissolved in .5-ml of distilled water, which resulted in an average dose of 95 mg/kg. Nine birds were tested for tonic immobility 15 min after injection, another nine 30 min postinjection, and the remaining eight birds were allowed to wait 60 min before being tested.

### *Results*

The temporal parameters of the tryptophan effect on tonic immobility are depicted in Figure 2. The apparent conversion of tryptophan into 5-HT is accomplished more rapidly than in rats, with effects becoming maximal 30 min after systemic injection. For purposes of analysis, again because of heterogeneity of variance, the data were subjected to a log transformation, and evidence for a statistically reliable between group difference was obtained, $F(2, 23) = 4.77, p < .025$. It is also interesting to note that scores from both the 30- and 60-min-postinjection groups are similar to those of the 55.8 and 139.5 mg/kg groups in Experiment 1 (see Figure 1).

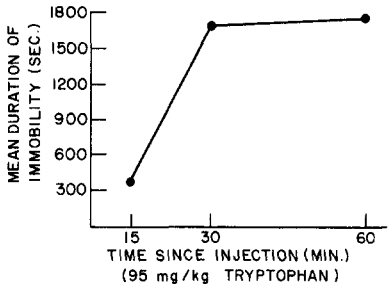


*Figure 2.* Mean duration of tonic immobility in chickens as a function of time since an injection of 95 mg/kg of tryptophan.

## Experiment 3

A behavioral change following tryptophan preloading does not, however, firmly establish that such changes are mediated by increases in brain 5-HT levels. Modigh (1973) addressed this issue, noting that tryptophan has alternative metabolic routes (e.g., tryptamine and niacin) which can confound any simple interpretation. Extrapolating from doses used by Dewhurst and Marley (1965), we designed the third experiment to assess the possible effects of tryptamine, another metabolic byproduct of tryptophan, on tonic immobility.

### *Method*

Twenty-four Production Red chickens were housed and maintained under the same conditions as employed previously. At approximately 3 wk of age the birds were randomly divided into three groups. Two groups received ip injections of tryptamine HCl dissolved in .5 ml of distilled water in amounts of either .31 or 1.24 mg. After testing, the birds were weighed and average doses were computed to be 2 mg/kg and 9 mg/kg, respectively. The third group received a control injection of distilled water. All birds were tested for tonic immobility 30 min following injection, and testing was conducted in the same manner as described in the previous experiments.

### *Results*

Data from the third experiment are summarized in Table 1. The between-groups differences failed to approach statistical significance, $F(2, 21) = .88$, and orthogonal comparisons were also nonsignificant.

## Experiment 4

Another, less direct means of affecting changes in plasma tryptophan is to restrict the dietary intake of this essential amino acid. Fernstrom and Wurtman (1971b) and Lytle, Messing, Fisher, and Phebus (1975) found that rats maintained on corn, which is low in tryptophan, showed diminished levels of brain serotonin. In an attempt to assess the presumed effect of decreased concentrations of brain 5-HT on tonic immobility, chicks in one group were fed a

Table 1
*Effect of Tryptamine HCl on the Duration (in Sec) of Tonic Immobility*

| Statistic | Water | Tryptamine (mg/kg) | |
|---|---|---|---|
| | | 2.06 | 9.02 |
| $\bar{X}$ | 667.50 | 348.63 | 459.88 |
| *SD* | 434.95 | 474.29 | 547.47 |

diet consisting exclusively of crushed corn and compared with controls maintained normally.

### *Method*

The subjects were 24 Production Red chickens obtained and maintained as before. At 1 wk of age the birds were randomly divided into two groups and placed in separate brooders. The birds in one group were fed crushed corn, supplemented with gravel to aid digestion. Chickens in the remaining condition continued to receive Purina Chick Chow and were also given access to gravel. At 18 days of age the birds from both groups were tested for tonic immobility and then weighed prior to being returned to the brooders.

### *Results*

The birds provided with a tryptophan-restricted diet of crushed corn evidenced a mean duration of immobility of only 47.5 sec compared with 359.3 sec for those receiving a balanced diet. In other words, birds maintained normally evidenced immobility reactions that averaged over seven times longer than those of birds placed on chronic corn consumption. An analysis of variance of these data yielded evidence of a significant difference between groups, $F(1, 22) = 6.35, p < .025$. As a consequence of the dietary differences, however, there were appreciable differences in body weight, with control birds weighing almost twice as much ($\bar{X}$ = 133 g) as the chicks fed crushed corn ($\bar{X}$ = 68 g).

## Experiment 5

Although a corn diet greatly diminished the duration of tonic immobility, with a mean of 47.5 sec, most birds still showed some degree of the reaction. Corn is poor in tryptophan but not completely lacking in this essential amino acid. A more complete depletion of endogenous tryptophan might have an even more pronounced effect. It would also follow that the opportunity to ingest a balanced diet after tryptophan depletion should serve to reinstate immobility scores. Therefore, birds were fed a specially prepared tryptophan-free food, tested for tonic immobility, and then given dietary replacement prior to being retested.

### *Method*

The subjects were 20 Production Red chickens obtained and maintained initially as in the previous experiments. At 2 wk of age the birds were randomly divided into two groups and housed in separate brooders. Birds in one group were continued on a normal diet, and those in the other group were fed a tryptophan-free powdered diet, containing a niacin supplement, obtained from Nutritional Biochemicals Corporation (Cleveland). Twelve days later both groups were tested for tonic immobility.

After testing, the birds deprived of tryptophan were given ad lib access to normal feed for seven additional days, and then both groups were retested for tonic immobility.

### *Results*

On the first test, birds that were maintained normally evidenced immobility reactions that averaged over 50 times longer than chicks that consumed tryptophan-free food, with mean durations of 507.4 sec and 9.5 sec, respectively, $F(1, 18) = 9.00, p < .01$. However, as was the case for birds maintained on corn, the tryptophan-free diet was associated with appreciable growth retardation. On the day of testing, control birds weighed an average of 386 g, while birds on the special diet averaged only 174 g.

Seven days following dietary replacement the differences in immobility were considerably reduced. Control birds evidenced the typical decrement in immobility as a result of repeated handling and testing (Nash & Gallup, 1976). However, after access to normal food, subjects previously deprived of tryptophan exhibited an increase in immobility that averaged over nine times that shown on the previous test. Chicks in the control group showed a mean duration of immobility of 154.1 sec,

while birds given remedial dietary replacement averaged 88.1 sec, and this difference failed to reach statistical significance ($F = .80$). Thus, the opportunity to ingest a balanced diet served at least partially to reinstate the immobility reaction.

It is interesting to note that in spite of the diminished immobility differences following access to normal food, appreciable weight differences still obtained between groups, with mean body weights on the second test of 570 versus 343 g, $F(1, 18) = 17.42$, $p < .001$. In an independent attempt to assess further the possible influence of weight, correlational analyses failed to reveal any significant relation between body weight and duration of immobility ($r = .12$ for controls and $-.11$ for those previously deprived of tryptophan).

## Discussion

These experiments show that systemic pretreatment with tryptophan reliably prolongs the duration of tonic immobility in chickens whereas dietary depletion greatly diminishes, if not eradicates, the reaction. On the other hand, access to normal food following tryptophan deprivation serves to partially reinstate the response. Taken together, our results provide a strong inferential basis for suggesting that altered levels of plasma tryptophan provide a means of achieving bidirectional control over the duration of tonic immobility in chickens and, in conjunction with previous drug work (e.g., Maser et al., 1975) and neuroanatomical (Klemm, 1971) and electrophysiological data (e.g., Aghajanian & Haigler, 1973), implicate specific serotonergic involvement. Unlike catecholaminergic systems, precursor availability seems to be a more important factor in determining the rate of serotonin synthesis (Fernstrom et al., 1974), and all the tryptophan manipulations in the present study were shown to have substantial effects on brain 5-HT concentrations (e.g., Fernstrom & Wurtman, 1971b).

That the effects are due to serotonin synthesis is also supported by several additional facts. In Experiment 3, intraperitoneal injections of tryptamine, an alternative metabolic by-product of tryptophan, were not found to affect tonic immobility. Niacin, still another tryptophan metabolite, also appears as an unlikely candidate, since vitamin supplements containing niacin were added to the tryptophan-free diet in the last experiment.

A possible objection to those experiments purporting to show a relation between diet and duration of immobility is the striking covariation between dietary depletion of tryptophan and body weight. Birds maintained on corn and those provided with tryptophan-free food evidenced appreciable weight losses. Comparable weight losses are typical for rats maintained on similar diets (e.g., Lytle et al., 1975). There are, however, several reasons why such changes are probably not responsible for the observed effects on tonic immobility. In the first place, 24-hr food deprivation has been found to have no effect on the reaction in chickens (Gallup & Williamson, 1972). Following 72 hr of food deprivation Rovee, Kaufman, and Lelek (Note 2) reported a decrease in tonic immobility at night but a slight nonsignificant increase during daylight hours. As shown in the fifth experiment, within-groups individual differences in weight do not correlate significantly with differences in immobility. Although access to a normal diet following tryptophan depletion served to reinstate the response and eliminate between group differences, substantial and highly significant weight differences persisted, which strongly suggests that weight per se is unimportant. Finally, it is intriguing to note that food deprivation may contribute to rather than detract from 5-HT depletion. Hollister, Breese, Kuhn, Cooper, and Schanberg (1976) suggested that food deprivation may produce deficiencies in 5-HT precursors and correlated changes in 5-HT metabolism; for example, they found that either 5-HTP or tryptophan injections blocked the potentiating effect of food deprivation on *d*-amphetamine-induced changes in activity in rats.

An alternative neurochemical account of tonic immobility attributes the reaction to a central cholinergic inhibitory system (Thompson, Piroch, Fallen, & Hatton,

1974). Thompson et al. found that scopolamine, an anticholinergic, abbreviated the duration of immobility in chickens whereas an attempt to block acetylcholinesterase with physostigmine prolonged the response. However, cholinergic effects do not necessarily mean that tonic immobility is mediated by the cholinergic system. Atropine, a relatively specific cholinergic blocker, has no effect on immobility in chickens even at unusually large doses (Hicks, 1975; Maser et al., 1975). In light of the parallel between raphe electrical activity and immobility durations, it is interesting to note that both atropine and scopolamine fail to affect raphe firing (Aghajanian et al., 1970). Any cholinergic influence on tonic immobility is further complicated by the fact that the effects of both scopolamine and physostigmine produce just the opposite results on tonic immobility in rabbits compared with chickens (Hatton, Woodruff, & Meyer, 1975), and in guinea pigs compared with ducks (Woodruff, Hatton, Frankl, & Meyer, 1976). Finally, scopolamine appears to have tranquilizing properties (e.g., Plotnik, Mollenauer, & Snyder, 1974), and tonic immobility is attenuated by procedures designed to reduce fear (see Gallup, 1974).

In a recent study Lytle et al. (1975) found that rats maintained on a tryptophan-poor diet showed diminished levels of brain 5-HT and increased responsiveness to electric shock, as measured by reduced flinch and jump thresholds. These investigators concluded that their results are "the first that relate behavioral changes that follow dietary manipulations directly to a change in a putative brain neurotransmitter" (p. 694). The present findings extend these and other effects of altered plasma tryptophan levels to a different behavior and suggest a fundamental dependence on brain 5-HT levels and/or raphe electrical activity in the modulation of tonic immobility in chickens.

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