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Biphasic Effects of Serotonin on Tonic Immobility in Domestic Fowl

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HENNIG, C. W. *Biphasic effects of serotonin on tonic immobility in domestic fowl.* PHARMAC. BIOCHEM. BEHAV. 12(4) 519–523, 1980.—In two experiments, groups of 2½ to 3 week old chickens were injected intravenously with various dosages of serotonin and tested for tonic immobility. Relatively low doses of serotonin produced significant increases in the duration of immobility, while high doses only produced slight decreases in duration. Serotonin also reduced the activity levels of chickens before testing for immobility and made the response easier to induce, but there were no apparent differences due to dosage for either of these behaviors. The effects by serotonin on the immobility response and activity level were found to persist for over two hours with little decrease in their potency. The data in this study are used to explain the contradictory effects produced by serotonin on immobility during past research and are discussed in terms of the importance of differential responding by prey during predatory encounters.

Tonic immobility Animal hypnosis Biphasic effects Serotonin Chickens Raphe nuclei

TONIC immobility (TI), also known as animal hypnosis and death feigning, is a dramatic state of profound motor inhibition that is reliably elicited by a brief period of physical restraint, typically induced by holding an animal down on a flat surface. The animal reacts by struggling and trying to escape, but after a few seconds these reactions subside and it assumes a motionless, catatonic-like posture which persists in the absence of further restraint. This response can last from a few seconds to several hours and is characterized by inhibition of movement, intermittant eye closure, waxy flexibility, hypertonicity of skeletal muscles, Parkinsonian-like tremors, and suppressed vocalization.

Tonic immobility has been demonstrated in animals from such diverse groups as insects, fish, amphibians, reptiles, birds, and mammals. Domestic fowl are often studied because they display a pronounced immobility response that is readily induced and easily quantifiable. Tonic immobility has proven especially sensitive to manipulations designed to affect fear, and has been conceptualized as the terminal defense reaction in a sequential series of distance-dependent predator defenses (for reviews see [4,19]).

There are a number of studies showing drug-related effects on tonic immobility. Most of these studies have sought to identify critical neurotransmitters underlying the immobility response. Both the cholinergic and adrenergic systems have been implicated with TI in several studies [12, 24, 25, 26, 27]. The strongest evidence, however, seems to favor serotonin (5-hydroxytryptamine, 5-HT) as the main neurochemical mediator for tonic immobility (for a review see [28]). In a broader sense, a number of investigators have already suggested that 5-HT may participate in various forms of behavioral inhibition such as freezing in a fear situation, passive avoidance prompted by punishment, and reduction of general locomotor activity [14, 15, 23]. A similar system may operate for tonic immobility. There are, however, still some questions that must be answered about serotonergic involvement with tonic immobility.

EXPERIMENT 1

There have been several studies that examined the effects of direct administration of serotonin in animals on the duration of tonic immobility, but the results have varied. Intravenous injections of 5-HT in young chickens produced a decrease in TI duration [17], whereas, they had no effect on TI in rabbits [7]. On the other hand, intraventricular administration of 5-HT in chickens produced an increase in TI duration [6], while a similar procedure with rabbits produced a decrease in duration of immobility [7]. These results have been attributed to differences in the drug's route of administration or the species used, but there were also other methodological differences, the most obvious of which were the differences in serotonin dosages that were employed. Wallnau and Gallup [28] suggest that 5-HT may affect tonic immobility via a mechanism in the raphe nuclei, since administration of 5-HT is known to decrease raphe firing [2]. However, it is now known that serotonin has a biphasic effect on raphe metabolism, with small doses of 5-HT producing a decrease in raphe activity, but larger doses having no apparent effect [13]. In addition, chlorpromazine, which is known to affect the serotonergic system, produced biphasic effects on TI duration that were dependent upon dosage [16]. It was

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decided, therefore, to assess the effect of various doses of 5-HT on the immobility response. There is some question, however, on how readily 5-HT will cross the blood-brain barrier. Therefore, young chickens were chosen as subjects, since their blood-brain barrier is not fully formed until after 3 or 4 weeks of age [22], so that simple intravenous injections of serotonin could be used. General activity will also be examined in order to determine whether any effects of 5-HT on TI can be generalized to other types of behavior.

METHOD

Animals

The subjects were 40 straight run Production Red chickens (*Gallus gallus*), $2^1/_2$ to 3 weeks of age, obtained from a local hatchery at one day of age and group-reared in commercial brooders under a 14 hr light cycle (6 a.m.–8 p.m.). Food (Purina Chick Chow) and water were continually available. On the day of the experiment the mean weight of the chicks in all groups was 150.8 g with a standard deviation of 19.3.

Procedure

On the day of the experiment, subjects were randomly selected from the brooder and divided into four groups of 10 subjects each. One group of chickens received intravenous (IV) injections of 0.3-cc saline. Each of the other three groups received comparable volumes of saline containing 0.034, 0.68, or 3.4 mg of serotonin creatinine sulfate. All injections were IV to the humeral wing vein (V. cutanea ulnaris). Only after the testing was completed were the subjects weighed, and an average dose of the drug calculated for the group. This procedure was used because handling alone can exert a profound effect on TI [5]. The average dosages of serotonin were 0.24, 4.72, and 21.8 mg/kg for the respective groups.

Immediately following injection, each subject was placed in a cardboard box, carried to the testing room, and allowed to remain there for 10 min. Then the bird was removed from the box, placed on a table, and its behavior was noted for 5 sec. If the bird changed position it was scored as active; if it stood or sat in place it was scored as passive. After this, the subject was picked up and quickly inverted on its right side, whereupon gentle restraint was maintained with both hands for 15 sec, at which time the experimenter slowly withdrew his hands and activated a stopwatch. While timing the reaction, the experimenter remained relatively motionless, seated about 0.5 m away from the subject and observed it with an indirect gaze. Any subject failing to remain immobile for at least 10 sec was given up to five successive 15-sec inductions in an attempt to elicit immobility, with a 30 sec intertrial interval between attempts. If the subject did not show immobility of at least 10 sec duration for any of the five attempted inductions, a duration score of zero was recorded. For those birds that did become immobile, the duration of TI was measured from the time of release until either the bird showed a spontaneous righting response and returned to its feet, or a maximum duration of 3600 sec had elapsed. Since a number of recent studies [8, 9, 20] have documented circadian rhythms in immobility duration, subjects were tested in random replications so that any possible circadian rhythm effects were evenly distributed across groups. Testing and data recording were performed by experimenters who were unaware of the drug injected in the subjects used in this study.

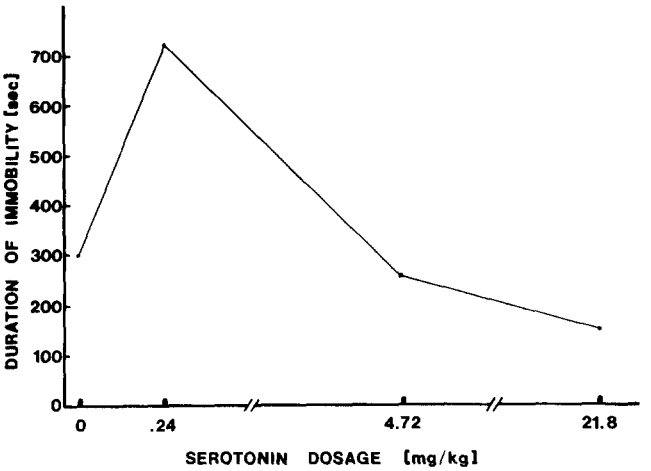

FIG. 1. Mean durations of tonic immobility as a function of average serotonin dosage.

RESULTS

Figure 1 depicts the dose response curve for serotonin and duration of tonic immobility. The lowest dose of 5-HT was found to enhance TI duration, while the highest dose may have produced a slight decrease in duration of immobility. Due to extreme skewness in the data, square root transformations were performed on all scores before statistical analysis and resulted in a normalization of data. An analysis of variance (ANOVA) performed on the transformed scores in this experiment revealed a significant overall difference between groups, $F(3,36)=3.16$, $p=0.0363$. Subsequent orthogonal contrasts revealed the mean duration of TI for the 0.24 mg/kg serotonin dose group to be significantly greater than the pooled means of the other three groups, $F(1,36)=8.36$, $p=0.0065$, however, the pooled means for the two highest 5-HT groups did not differ significantly from the mean for the control group. The number of inductions required to produce immobility are shown in Table 1. As can be seen, those subjects that received any amounts of 5-HT seemed more susceptible to tonic immobility than those that received saline. This result was supported by an orthogonal contrast which showed that the mean number of inductions needed for the control group were significantly greater than the pooled means for the three 5-HT groups, $F(1,36)=5.71$, $p=0.0222$. There were no apparent differences in susceptibility between the three 5-HT groups. The general activity levels of the chickens before immobility also seem to depend on the presence of serotonin. As can be seen in Table 1, more subjects became passive after receiving injections of serotonin. This was especially true for those chickens that received the largest dose of the drug. An ANOVA performed on this data revealed a significant overall difference between groups on the number of animals that were active, $F(3,36)=4.30$, $p=0.0108$. Subsequent orthogonal contrasts revealed that the mean for the control group differed significantly from the pooled means for the three 5-HT groups, $F(1,36)=7.35$, $p=0.0102$, and the mean for the highest 5-HT dose group differed significantly from the pooled means for the lower 5-HT dose groups, $F(1,36)=5.57$, $p=0.0238$.

EXPERIMENT 2

The first experiment revealed that low doses of 5-HT

TABLE 1

ACTIVITY STATE BEFORE IMMOBILITY AND THE NUMBER OF INDUCTIONS REQUIRED TO PRODUCE IMMOBILITY IN EXPERIMENTS 1 AND 2

Experiment 1

Dependent variable		Groups: Control	0.24 mg/kg Serotonin	4.72 mg/kg Serotonin	21.8 mg/kg Serotonin
Active	Yes	7	4	4	0
	No	3	6	6	10
Mean number of inductions		1.70	1.00	1.00	1.10

Experiment 2

Dependent variable		Postinjection time periods: 10 min Cont	10 min 5-HT	60 min Cont	60 min 5-HT	120 min Cont	120 min 5-HT
Active	Yes	6	2	6	4	8	3
	No	3	7	3	5	1	4
Mean number of inductions		2.78	2.11	2.56	1.56	2.67	1.11

produced increases in the duration of tonic immobility, but this effect was not as dramatic as those produced by other drugs [11, 12, 16, 17]. Moreover, a recent study [12] has shown that injections of 5-HT metabolites, melatonin and 5-hydroxyindole acetic acid, produce opposite effects on TI duration at different times after injection. Therefore, the present experiment in this study was designed to replicate the previous increases in immobility with low doses of 5-HT and determine the time course of this phenomenon over longer postinjection periods, in order to find the optimal time after injections of serotonin for immobility testing.

METHOD

Animals

The subjects were 54 straight run Production Red chickens, 2 1/2 to 3 weeks of age, obtained and maintained as in the previous experiment. The only appreciable difference was that the mean weight of the chicks in all groups on the day of testing was 126.3 g with a standard deviation of 21.8.

Procedure

On the day of the experiment, subjects were randomly assigned to six groups of 9 subjects each. Three groups received IV injections of 0.3-cc of saline, while the other three groups received comparable volumes containing 0.3 mg of serotonin creatinine sulfate. This produced mean 5-HT doses calculated to be 2.4 mg/kg. Subjects in one control and one 5-HT group were tested 10 min postinjection, as previously described in the first experiment, while a second control and drug group were tested 60 min postinjection and the last two groups were tested at 120 min. Subjects were housed in glass terraria for these prolonged time periods and were maintained in cardboard boxes only for the 10 min immediately prior to testing. All other procedures were the same as in the previous experiment.

RESULTS

The mean durations of tonic immobility for both 5-HT and control groups at 10, 60, and 120 min postinjection are shown in Fig. 2. As can be seen, the mean durations of TI are considerably longer for the 5-HT groups, regardless of time after injections, although there seems to be a slight decrease in the effect by 5-HT at two hours after injection. After a square root transformation, a 2-way factorial ANOVA was performed on these data, with drug condition and postinjection time as independent variables. This analysis revealed a significant main effect due drug condition, $F(1,48)=4.27$, $p=0.0442$, with serotonin producing significantly longer durations of TI than saline injections. No other factors produced a significant difference. The number of inductions required to produce TI are shown in the lower part of Table 1. As can be seen, all the 5-HT groups were more susceptible to TI than their controls. This difference was supported by the results of a 2-way factorial ANOVA which revealed a significant main effect due drug condition, $F(1,48)=6.57$, $p=0.0136$. There were no significant effects due time since injection or the interaction of variables. Similar results were found with the general activity levels shown by the chickens before TI was induced. As can be seen in Table 1, fewer subjects in each of the 5-HT groups were active than in their respective control groups. This difference was supported by the results of a 2-way factorial ANOVA which revealed a significant main effect due drug condition, $F(1,48)=10.08$, $p=0.0026$, but no appreciable effect by any other factors.

DISCUSSION

The present study has vividly demonstrated that injec-

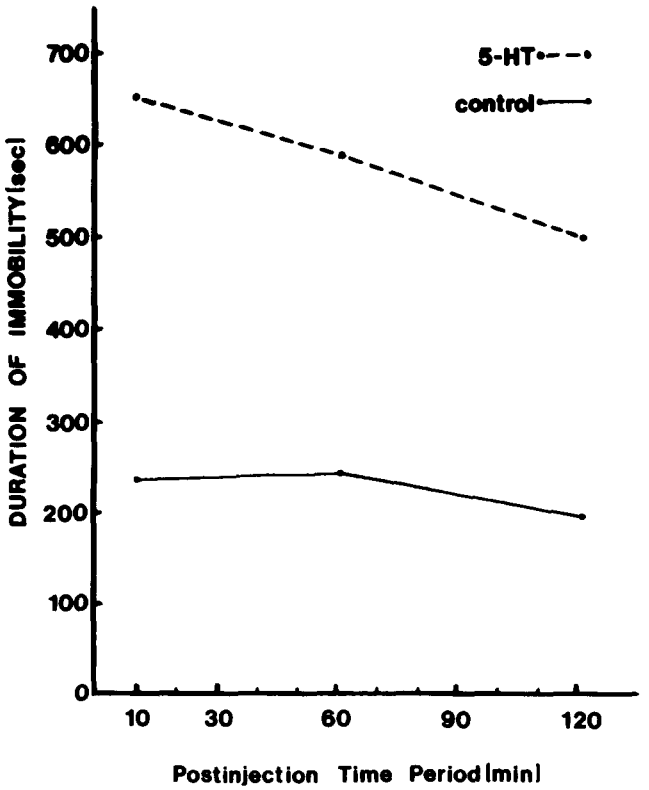

FIG. 2. Mean durations of tonic immobility as a function of time after injection (10, 60 or 120 min) for groups that received either saline or serotonin (average dose of 2.4 mg/kg).

tions of serotonin produce a biphasic effect on duration of TI in chickens. Small doses of 5-HT produce an increase in immobility duration, while larger doses may have no effect or might even produce a slight decrease in duration of immobility. These results help to explain conflicting reports from previous research. When serotonin was administered centrally, it produced an increase in TI duration in chickens [6], while a decrease in immobility duration occurred in rabbits [7]. This might seem to suggest some kind of reversal in the effect of centrally administered 5-HT on TI duration, depending on which species was utilized, but the latter study used almost 10 times larger doses of 5-HT than the former study. Relatively large intravenous injections of serotonin in chickens were also found to produce significant decreases in TI duration [17]. Intravenous injections of large doses of 5-HT, however, had no apparent effect on TI in rabbits [7] or anoles [11], but this may be due to the fact that serotonin is not known to readily cross the blood-brain barrier. The present study obtained an increase in TI duration with small doses of 5-HT and a trend toward a decrease in duration of TI with the highest dose of serotonin, although the latter change did not reach statistical significance. Taken together, these findings suggest that large doses of 5-HT have an inhibitory effect on TI duration, while low doses of serotonin have a potentiating effect on this behavior. Thus, these differences could just as easily be explained as another example of the biphasic effect by 5-HT on duration of TI, regardless of differences in the route of administration of the drug. This is not totally unexpected since biphasic effects on several other behaviors have been noted after central administration of serotonin in rats. Small doses of 5-HT have decreased motor activity and reactivity to stimuli, caused drowsiness, and produced slow-wave sleep, while higher doses of 5-HT have induced hyperactivity, tremors, and EEG excitation [13,21]. In addition, serotonin has also produced initial central depression and sleep in chickens, followed by central nervous system excitation and behavioral arousal [3]. Moreover, central administration of low doses of 5-HT in rats produced a decrease in the firing rate [1,2] and metabolism [13] of raphe neurons, while higher doses had no apparent effect [13], and much greater peripheral doses even increased raphe activity [18]. Thus, one can see that 5-HT appears to act on both the raphe neurons and tonic immobility in a parallel manner, supporting the hypothesis by Wallnau and Gallup [28] of a midbrain-raphe control system for tonic immobility. However, although TI may be controlled via this type of central nervous system mechanism, peripheral influences by 5-HT on TI cannot be completely ruled out.

The present study also demonstrated the importance of 5-HT involvement with TI in several other ways. It showed that the increase in TI duration with low doses of 5-HT persisted for prolonged periods. Serotonin also increased an animal's susceptibility to TI over a similar period. It reduced the number of inductions required to produce the immobility response. This result was similar to the effect by 5-HTP on susceptibility to TI in anoles [11]. This effect does not seem to be biphasic and suggests that susceptibility to TI and the duration of the immobility response may have slightly different mechanisms. These results also show the importance of 5-HT to TI, since most other drugs that affect duration of TI are not known to influence the number of inductions required to produce the immobility response [10, 11, 12, 17, 26]. Serotonin had a similar type of effect on general activity in chickens, without any indication of a biphasic quality, although such a result has been noted with central administration of 5-HT in rats [13,21]. Moreover, serotonin has been shown to produce sleep in chickens, which was later followed by arousal [3]. The present lack of a biphasic effect by 5-HT on general activity in chickens may be due methodological differences or the use of too low a dose of serotonin. However, these differential effects by 5-HT on various behaviors show that one should be aware that not all behaviors controlled by the serotonergic system may be affected by injections of 5-HT in the same manner and that generalizations should be restricted until more behaviors have been examined.

Another point of interest in the study of the serotonergic system's involvement with TI is the immobility response's presumed relation to fear and threat of predation [4,19]. One might ask why a biphasic effect by 5-HT on TI would evolve. The reason for this might be that it was highly advantageous to have a predator defense system where an animal sometimes remains immobile for long periods and at other times for only short periods, so that a predator could not learn to predict which response would occur. Similarly, it also makes sense that an animal's initial susceptibility to TI would not be controlled by the same system, since TI is only utilized as a last resort and wide differences in responding to restraint by predators might be maladaptive.

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