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Tonic Immobility: Effects of Dopamine Receptor Blockade and Stimulation[1]

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WALLNAU, L. B., C. L. CARNRIKE, JR. AND G. I. DEWEY. *Tonic immobility: Effects of dopamine receptor blockade and stimulation.* PHARMAC. BIOCHEM. BEHAV. **10**(2) 177–181, 1979.—Blockade of dopaminergic receptors by haloperidol enhanced the duration of tonic immobility in chickens. Apomorphine, a dopamine receptor agonist, produced short durations. Apomorphine also produced an increase in stabilimeter activity. These data suggest dopaminergic involvement in tonic immobility, and support a competing response interpretation of the apomorphine effect.

Tonic immobility Catalepsy Locomotor activity Haloperidol Apomorphine Dopamine Serotonin Chickens

TONIC immobility (TI) is a naturally occurring response which is elicited by brief physical restraint. Under laboratory conditions, one simply holds the animal on a flat surface. Initially the animal struggles and attempts to escape, but these responses subside and are followed by a motionless, catatonic-like posture which persists after the animal is released. TI is displayed by animals from diverse groups, such as avians, reptiles, fish, insects, crustaceans, and mammals [15]. Domestic fowl are often studied because they display a pronounced response that is easily induced and quantified. In addition to inhibition of movement, the response is also characterized by waxy flexibility, tremors of the extremities, hypertonicity of skeletal muscles, intermittent eye closure, decreased vocalization, and mydriasis [15,16]. Physiological changes during TI have also been observed and include changes in EEG patterns, respiration, heart rate and core temperature [23,29].

The duration of TI is directionally sensitive to manipulations which are designed to alter central serotonergic function. Specifically, there appears to be an inverse relationship between a drug's effect on TI duration and its effect on the firing rate of central serotonergic neurons of the midbrain raphe nuclei [37]. For example, drugs which are known to inhibit raphe activity in mammals (e.g., LSD, pargyline, tryptophan, and centrally administered serotonin) prolong immobility, while drugs which activate raphe neurons (e.g., amphetamine, peripherally administered serotonin) attenuate the reaction [1, 2, 3, 4, 7, 14, 17, 25, 26]. Wallnau and Gallup [37] have proposed a midbrain-raphe model of TI duration, in which the inhibition of raphe neurons, possibly reflecting increased synaptic function of serotonin (5-HT), is associated with enhanced immobility durations, while an increase on raphe firing is correlated with short reactions.

Evidence for dopaminergic involvement in TI is scant. Dopamine (DA) and L-dopa fail to alter TI (unpublished data). While dose and time parameters, as well as route of administration may account for such failures, these results are surprising in light of the apparent role of the dopaminergic and extrapyramidal systems in psychomotor function [11,31]. Furthermore, the highest concentrations of both DA and 5-HT in the avian brain are located in the anterior nucleus basalis, an area considered to be homologous to the mammalian corpus striatum [21]. It is interesting that in mammals, manipulation of DA receptors (e.g., haloperidol, apomorphine) have been found to interact with a serotonergic mechanism. For example, 5-HT depletion by PCPA or raphe lesions blocks haloperidol-induced catalepsy [9, 19, 24]. Furthermore, midbrain raphe lesions potentiate apomorphine-induced locomotor activity in rats [18]. Serotonergic modulation of apomorphine effects has also been observed in avians [8].

Given the existing support for serotonergic involvement in TI and the role of both 5-HT and DA in psychomotor function, the present study examined the effect of both DA receptor blockade and stimulation on TI duration.

EXPERIMENT 1

Haloperidol, a specific DA receptor antagonist [5], produces cataleptic behavior in mammals [9,19]. In an attempt to examine the role of a dopaminergic system in TI, varying doses of haloperidol were administered to domestic fowl.

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METHOD

Animals

Fifty-two, straight-run, Production Red chickens were obtained at two days of age from a commercial hatchery (Welp Inc.). The animals were group housed in commercial thermostatically heated brooders. Purina chick chow and water were continuously available. A daily photoperiod consisted of artificial illumination from 8 a.m. to 10 p.m. Since habituation and familiarization with humans attenuate TI [28], the presence of humans in the colony room was restricted to routine daily maintenance.

Procedure

At eighteen days of age, thirty animals were randomly assigned to one of three groups. Animals were weighed and received either 1.0 or 2.0 mg/kg of haloperidol (Haldol, McNeil) intraperitoneally, or an equivalent volume (0.8 ml/kg) of distilled water. Animals were then placed in cardboard holding boxes (25×30×50 cm) for the duration of the injection-test interval. Each box was coded by number so that the persons conducting the tests were kept uninformed of group designations.

Thirty minutes following injection, each animal was carried to a room in the holding box where it was tested individually. The bird was removed from the box and manually restrained on its right side on a table for 15 seconds. The duration of TI was timed with a stopwatch from the moment the experimenter released the subject until it righted itself and rose to its feet. If the initial induction attempt did not elicit TI, the animal was restrained again 60 seconds later. During the intertrial interval the animal was returned to the holding box. Induction attempts were repeated in this fashion until an animal exhibited TI, or until there had been five unsuccessful induction attempts. Birds that received five inductions and still showed no reaction were given a duration of zero seconds. All testing was conducted between 10 a.m. and 3 p.m., with each group equally represented across time of testing.

In order to control for the possible effect of the vehicle for haloperidol, twenty-two additional animals were subsequently assigned to one of two groups. At 24 days of age, half of the animals received 0.8 ml/kg of the lactic acid vehicle used for haloperidol or an equivalent volume of distilled water. The vehicle contained 0.002 ml of lactic acid (USP) per ml of distilled water and two preservatives, 0.4 mg/ml of methylparaben and 0.05 mg/ml of propylparaben. Animals were tested as described above, 30 min following injection.

RESULTS

Figure 1 depicts the effect of haloperidol on TI duration. Due to skew and heterogeneity of variance, $F\max=8.48$, $p<0.05$, a square root transformation was performed prior to data analysis [22]. Analysis of variance revealed an overall effect of haloperidol, $F(2,27)=7.84$, $p<0.003$. An analysis of the trend components revealed significant linear, $F(1,27)=9.74$, $p<0.005$, and quadratic, $F(1,27)=5.93$, $p<0.03$, effects. Post hoc comparisons by a Newman-Keuls test revealed the nature of these trends. The control group was significantly lower than the 1 mg/kg ($p<0.01$) and 2 mg/kg ($p<0.05$) groups. There was no difference between either group of animals that received haloperidol. Thus, both doses of haloperidol were equally effective in potentiating TI duration. Regarding susceptibility to TI, nearly all of the animals in each group displayed TI on the first induction. No systematic effects for number of inductions were found in the remaining experiments.

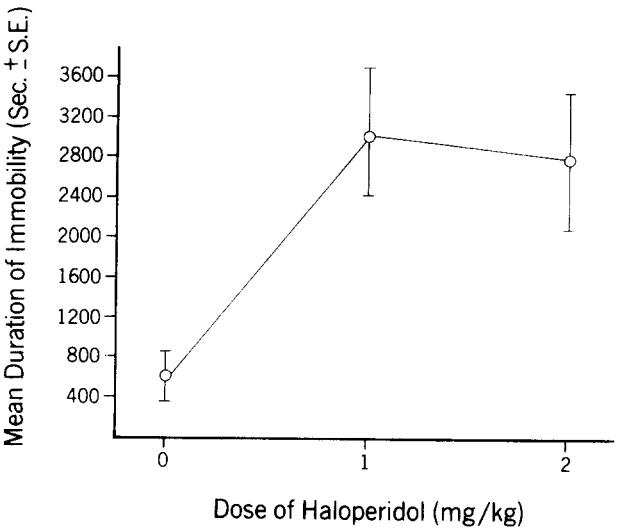

FIG. 1. The effect of varying doses of haloperidol on the duration of tonic immobility.

The additional distilled water- and vehicle-injected control groups displayed mean durations of 817.64 and 624.55 sec respectively. Due to heterogeneity of variance, $F\max=5.51$, $p<0.05$, a square root transformation was performed. Analysis of variance did not yield a significant effect for groups, $F<1$. Thus, the lactic acid vehicle had no effect on TI duration.

EXPERIMENT 2

The results of the previous experiment suggest that DA receptor blockade by haloperidol may enhance TI duration. As a partial replication of this finding, and to assess its time course, the effect of haloperidol was examined at several different post-injection intervals.

METHOD

Animals

Thirty straight run Production Red chickens were obtained and cared for as described in the previous experiment.

Procedure

At 17 days of age, animals were randomly assigned to one of three groups. All animals were weighed and received intraperitoneal injections of 2 mg/kg of haloperidol. Injection volumes were 1.6 ml/kg. Animals were then tested for TI either 15, 30, or 45 min following injection. The test procedures were identical to those reported in Experiment 1.

RESULTS

Figure 2 depicts the time course for haloperidol. To alleviate skew and heterogeneity of variance, $F\max=15.43$,

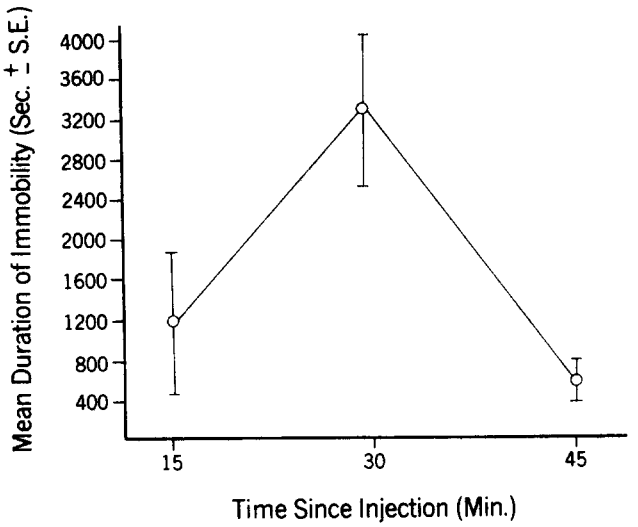

FIG. 2. The effect of haloperidol on tonic immobility duration as a function of injection-test interval.

$p<0.01$, a square root transformation was performed. Analysis of variance revealed a significant overall effect, $F(2,27)=7.29$, $p<0.003$. A trend analysis revealed that only the quadratic component was significant, $F(1,27)=14.41$, $p<0.001$. This effect was confirmed by a Newman-Keuls test. Both the 15 min and 45 min groups differed from the 30 min group ($p<0.01$ in each case), while there was no difference between the two former groups. Thus, at the times tested, haloperidol had optimal effects at 30 min following injection.

Since manipulations designed to increase fear potentiate TI [15,16], one might interpret these data in terms of the decay of fear associated with the injection procedure. However, this interpretation is precluded by the finding that fear manipulations enhance TI to the extent they are presented in close temporal proximity to response elicitation [27]. Fear effects on TI duration are greatly diminished when testing occurs more than 15 minutes after pretreatment with painful stimulation [27]. Thus if fear had been elicited by the injection procedure and consequently altered response duration, the 15 min group instead should have displayed enhanced durations. To the contrary, the post-hoc analysis revealed that there was no difference between the 15 and 45 min groups, but these groups in turn differed from the 30 min group.

EXPERIMENT 3

The results of the first two experiments suggest that DA receptor blockade by haloperidol greatly enhances TI. If this effect reflects dopaminergic involvement, then apomorphine, the DA receptor agonist [6], should substantially reduce TI duration. The present experiment examined the effect of varying doses of apomorphine on TI.

METHOD

Animals

Thirty-six straight run Production Red chickens were housed and maintained as described in Experiment One.

Procedure

At twenty days of age, animals were randomly assigned to one of four groups. Animals were weighed and then injected intraperitoneally with either 0.5, 1.0, or 3.0 mg/kg of apomorphine HCL (Lilly). Control subjects received an equivalent volume (1 ml/kg) of distilled water. Since apomorphine has been reported to alter behavior in avians just minutes after injection [8, 12, 30], subjects were tested for TI 2 min following injection. The test procedures described in Experiment 1 were employed.

RESULTS

The means and standard errors are depicted in Table 1. A square root transformation was performed to reduce skew and heterogeneity of variance, $F\max=296.83$, $p<0.01$. Analysis of variance yielded a significant overall effect for apomorphine, $F(3,32)=9.47$, $p<0.001$. A trend analysis revealed significant linear, $F(1,32)=5.96$, $p<0.025$, and quadratic, $F(1,32)=21.26$, $p<0.001$, effects. A Newman-Keuls post hoc test revealed that the control group displayed longer durations than each of the apomorphine groups ($p<0.01$, in each case), while there were no differences among the remaining groups. Thus, all doses of apomorphine produced abbreviated durations of tonic immobility.

TABLE 1

THE EFFECT OF APOMORPHINE HCL ON THE DURATION OF TONIC IMMOBILITY (IN SEC)

	Dose (mg/kg)			
	0	0.5	1.0	3.0
Mean	773.78	115.44	41.44	128.67
Standard Error	230.93	39.29	13.40	37.72

EXPERIMENT 4

The first three experiments provide support for dopaminergic involvement in TI. These data are consistent with the well documented dopaminergic involvement in psychomotor behavior (e.g., [31]). Specifically, DA receptor stimulation by apomorphine has been found to enhance locomotor activity and stereotyped behavior in both mammals [13, 18, 32] and avians [8, 12, 30]. It is possible therefore, that a competing response interpretation may account for the effects of DA receptor manipulations on TI. That is, DA receptor blockade may suppress locomotor activity and eliminate responses that would otherwise compete with TI. On the other hand, DA receptor stimulation may activate locomotor activity and stereotyped behavior which in turn competes with TI. Since a competing response interpretation is in part predicated on the presence of elevated locomotor activity following DA receptor stimulation, the effect of apomorphine on stabilimeter activity was examined.

METHOD

Animals

Eighteen straight run Production Red chickens were obtained, housed, and fed as described in the first experiment.

Apparatus

Locomotor activity was measured with a BRS/LVE activity platform (Model 124-04) which provides pulses when the platform slides horizontally in any direction and strikes a plumb. A transparent plastic compartment (20×23.5×21.5 cm) with a grid floor was placed on the activity platform for containing animals during the test. The entire apparatus was leveled and situated in a lighted and ventilated, sound-attenuated chest which was equipped with a small observation window. The activity platform was calibrated so that a pulse was recorded when the platform moved 3 mm in the horizontal plane. Activity counts were tallied with an electromechanical counter driven by a pulse former.

Procedure

At 23 days of age, animals were randomly assigned to one of two groups. Half of the animals received intraperitoneal injections of 3.0 mg/kg of apomorphine HCL, while the remaining animals received an equivalent volume (1 ml/kg) of distilled water. One min after injection, animals were placed in the activity compartment and activity was recorded for ten 1 min blocks. During the test session, informal notes were taken of the behavior of all animals.

RESULTS

The mean activity counts for the apomorphine and control groups were 579.11 and 10.0 respectively. Analysis of variance of the raw data revealed a significant effect for the drug condition, $F(1,16)=13.44, p<0.003$. Thus, apomorphine elevated locomotor activity. There was no effect for time blocks ($F<1$) nor was there a drug × time block interaction ($F<1$) Informal observations indicated that hyperactivity was accompanied by loud chirping, intense bouts of stereotyped pecking, and crouching which often precedes flight. These behaviors were observed within minutes of injection and are in agreement with other reports [8,30].

DISCUSSION

Haloperidol, a DA receptor blocker, greatly enhanced TI duration. This finding is consistent with reports of haloperidol-induced catalepsy [9,19], and the viewpoint that TI may provide a laboratory model of catatonia or catalepsy [16]. Other cataleptigenic agents have been observed to alter TI. Cholinergic agonists, for example, produce a cataleptic state in rats [10] and enhance TI duration in avians [35,38]. There is, however, a peculiar mammalian-avian reversal of cholinergic effects on TI ([38], see [37]). Morphine, which induces catalepsy in both rats [10] and chickens [33] is also a potent agonist of TI at sub-cataleptic doses [20]. It should be noted that at the doses employed in Experiments 1 and 2, no animal receiving haloperidol exhibited catalepsy spontaneously. In Experiment 2, haloperidol had an optimal effect 30 min following injection. It is seemingly paradoxical that at 45 min there was no haloperidol enhancement of TI, since the mean of the 30 min group (3266.7 sec) greatly overlapped the 15 min difference in injection-test intervals between these two groups. It is possible that the amount of DA receptor blockade at test time is an important variable and that another mechanism is also involved which participates in the maintenance of TI duration.

The results of Experiment 3 bolster the interpretation of DA participation in tonic immobility. Apomorphine was found to abbreviate TI at all doses. Apomorphine was also found to greatly enhance locomotor activity, a result which confirms other findings [12, 18, 30]. Animals also exhibited stereotyped behavior such as pecking and loud chirping following apomorphine injections. These findings indirectly support a competing response interpretation. That is, following apomorphine pretreatment, motor behavior is activated which in turn produces responses which compete with TI. In light of these results, it is interesting that amphetamine also reduces TI duration [7]. Amphetamine has been reported to increase locomotion in chickens [34,36], however, a competing response interpretation of the amphetamine effect is clouded by the failure to find changes in stabilimeter activity at doses which abbreviate immobility [7].

The serotonergic system has been found to influence haloperidol-induced catalepsy [9, 19, 24] and apomorphine effects in both rats and avians [8,18]. In light of the serotonergic involvement in TI [37] and the apparent involvement of a central dopaminergic mechanism, it would be interesting to assess the effects of possible serotonergic and dopaminergic interactions on tonic immobility.

REFERENCES

1. Aghajanian, G. K. Influence of drugs on the firing of serotonin-containing neurons in brain. *Fedn. Proc.* **31**: 91–96, 1972.
2. Aghajanian, G. K., W. E. Foote and M. H. Sheard. Lysergic acid diethylamide: Sensitive neuronal units in the midbrain raphe. *Science* **161**: 706–708, 1968.
3. Aghajanian, G. K., A. W. Graham and M. H. Sheard. Serotonin-containing neurons in brain: Depression of firing by monoamine oxidase inhibitors. *Science* **169**: 1100–1102, 1970.
4. Aghajanian, G. K. and H. J. Haigler. Direct and indirect actions of LSD, serotonin and related compounds on serotonin-containing neurons. In: *Serotonin and Behavior*, edited by J. Barchas and E. Usdin. New York: Academic Press, 1973, pp. 263–266.
5. Andén, N. E., S. C. Butcher, H. Corrodi, K. Fuxe and U. Ungerstedt. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur. J. Pharmac.* **11**: 303–314, 1970.
6. Andén, N. E., A. Rubenson, K. Fuxe and T. Hokfelt. Evidence for dopaminergic receptor stimulation by apomorphine. *J. Pharm. Pharmac.* **19**: 627, 1967.
7. Boren, J. and G. G. Gallup, Jr. Amphetamine attenuation of tonic immobility. *Physiol. Psychol.* **4**: 429–432, 1976.
8. Cheng, H. C. and P. Long. Dopaminergic nature of apomorphine-induced pecking. *Eur. J. Pharmac.* **26**: 313–320, 1974.
9. Costall, B., D. H. Fortune, R. J. Naylor, C. D. Marsden and C. Pycock. Serotonergic involvement with neuroleptic catalepsy. *Neuropharmacology* **14**: 859–868, 1975.
10. Costall, B. and R. J. Naylor. Neuroleptic and non-neuroleptic catalepsy. *Arzneimittel-Forsch.* **23**: 674–683, 1973.
11. Creese, I. and S. D. Iverson. The pharmacological and anatomical substrates of the amphetamine response in the rat. *Brain Res.* **83**: 419–436, 1975.
12. De Lanerolle, N. C. and O. M. Youngren. Chick vocalization and emotional behavior influenced by apomorphine. *J. comp. physiol. Psychol.* **92**: 416–430, 1978.
13. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10**: 316–323, 1967.

14. Foote, W. E., M. H. Sheard and G. K. Aghajanian. Comparison of effects of LSD and amphetamine on midbrain raphe units. *Nature* **222:** 567–569, 1969.
15. Gallup, G. G., Jr. Animal hypnosis: Factual status of a fictional concept. *Psychol. Bull.* **81:** 836–853, 1974.
16. Gallup, G. G., Jr. and J. D. Maser. Tonic immobility: Evolutionary underpinnings of human catalepsy and catatonia. In: *Psychopathology: Experimental Models*, edited by J. D. Maser and M. E. P. Seligman. San Francisco: Freeman, 1977.
17. Gallup, G. G., Jr., L. B. Wallnau, J. L. Boren, G. J. Gagliardi, J. D. Maser and P. H. Edson. Tryptophan and tonic immobility: Effects of dietary and systemic manipulations. *J. comp. physiol. Psychol.* **91:** 642–648, 1977.
18. Grabowska, M. Influence of midbrain raphe lesion on some pharmacological and biochemical effects of apomorphine in rats. *Psychopharmacologia* **39:** 315–322, 1974.
19. Gumulka, W., W. Kostowski and A. Czlonkowski. Role of 5-HT in the action of some drugs affecting extrapyramidal system. *Pharmacology* **10:** 363–372, 1973.
20. Hicks, L. E., J. D. Maser, G. G. Gallup, Jr. and P. H. Edson. Possible serotonergic mediation of tonic immobility: Effects of morphine and serotonin blockade. *Psychopharmacologia* **42:** 51–56, 1975.
21. Juorio, A. V. and M. Vogt. Monamines and their metabolites in the avian brain. *J. Physiol.* **189:** 489–518, 1967.
22. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont, Calif.: Brooks Cole, 1968.
23. Klemm, W. R. Neurophysiologic studies of the immobility reflex ("animal hypnosis"). In: *Neuroscience Research*, Vol. 4, edited by S. Ehrenpreis and O. C. Solnitzky. New York: Academic Press, 1971.
24. Kostowski, W., W. Gumulka and A. Czlonkowski. Reduced cataleptogenic effects of some neuroleptics in rats with lesioned midbrain raphe and treated with p-chlorophenylalanine. *Brain Res.* **48:** 443–446, 1972.
25. Maser, J. D., G. G. Gallup, Jr. and L. E. Hicks. Tonic immobility in chickens: Possible involvement of monamines. *J. comp. physiol. Psychol.* **89:** 319–328, 1975.
26. Mosko, S. and B. L. Jacobs. Effect of peripherally administered serotonin on the neuronal activity of midbrain raphe neurons. *Brain Res.* **79:** 315–320, 1974.
27. Nash, R. F. and G. G. Gallup, Jr. Effect of different parameters of shock on tonic immobility. *Behav. Meth. Res. Instrum.* **7:** 361–364, 1975.
28. Nash, R. F. and G. G. Gallup, Jr. Habituation and tonic immobility in domestic chickens. *J. comp. physiol. Psychol.* **90:** 870–876, 1976.
29. Nash, R. F., G. G. Gallup, Jr. and D. A. Czech. Psychophysiological correlates of tonic immobility in the domestic chicken *(Gallus gallus)*. *Physiol. Behav.* **17:** 413–418, 1976.
30. Osiude, G. and P. O. Adejoh. Effects of apomorphine and its interaction with other drugs in the domestic fowl. *Eur. J. Pharmac.* **23:** 56–66, 1973.
31. Papeschi, R. Dopamine, extrapyramidal system, and psychomotor function. *Psychiat. neurol. Neurochir.* **75:** 13–48, 1972.
32. Rotrosen, J., M. B. Wallach, B. Angrist and S. Gershon. Antagonism of apomorphine-induced stereotype and emesis in dogs by thioridazine, haloperidol and pimozide. *Psychopharmacologia* **26:** 185–194, 1972.
33. Schneider, C. Effects of morphine-like drugs in chicks. *Nature* **191:** 607–608, 1961.
34. Spooner, C. E. and W. D. Winters. Neuropharmacological profile of the young chick. *Int. J. Neuropharmac.* **5:** 217–236, 1966.
35. Thompson, R. W., J. Piroch, D. Fallen and D. Hatton. A central cholinergic inhibitory system as a basis for tonic immobility ("animal hypnosis") in chickens. *J. comp. physiol. Psychol.* **87:** 507–512, 1974.
36. Wallach, M. B., E. Friedman and S. Gershon. Behavioral and neurochemical effects of psychotomimetic drugs in neonate chicks. *Eur. J. Pharmac.* **17:** 259–269, 1972.
37. Wallnau, L. B., and G. G. Gallup, Jr. A serotonergic, midbrain-raphe model of tonic immobility. *Biobehav. Rev.* **1:** 35–43, 1977.
38. Woodruff, M. L., D. C. Hatton, M. B. Frankl and M. E. Meyer. Effects of scopolamine and physostigmine on tonic immobility in ducks and guinea pigs. *Physiol. Psychol.* **4:** 198–200, 1976.