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Effects of Serotonergic Agents on the Transport Response in Rats

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WILSON, C. AND M. PULIDO. *Effects of serotonergic agents on the transport response in rats.* PHARMACOL BIOCHEM BEHAV **66**(3) 541–545, 2000.—In two experiments, researchers investigated the effects of manipulating serotonin systems on the transport response and dorsal immobility response in developing rats. In Experiment 1, administration of ketanserin and cinanserin, but not metergoline, suppressed the transport response in 23-day-old rats. These agents were without effect on dorsal immobility durations. In Experiment 2, administration of quipazine to 30-, 40-, and 50-day-old rats resulted in significant increases in transport response intensities and dorsal immobility durations. Results are discussed with respect to the nature of the transport response. © 2000 Elsevier Science Inc.

Quipazine Metergoline Cinanserin Ketanserin Transport response

IN developing animals, emergence of serotonergic (5-HT) systems have traditionally been linked with a suppression of locomotor activity (5,6,9,10). This generalization now appears unwarranted (7), for manipulating 5-HT systems produces a variety of effects on behavior, many of which appear to be age dependent. For example, blocking these systems suppresses suckling in neonatal rats (20) but reinstates that behavior in slightly older animals (22). Stimulating the systems increases mouthing, forward locomotion, and forelimb paddling in neonates but suppresses peak activity in 14- to 18-day-old rats (9). Finally, administration of the 5-HT agonist 2-(1-Piperazinyl) quinoline maleate (quipazine) induces hindlimb stepping in spinally-transected infant rats, allowing the possibility that 5-HT neurons may activate hindlimb motor circuits (12), and decreases startle reactivity in adult rats (14).

In considering age-related differences with administration of 5-HT agents, Ristine and Spear (16) proposed that early-developing portions of 5-HT systems may mediate specific behaviors critical to a young organism's survival. As more adult-like behaviors emerge, these early functions of 5-HT may be suppressed. Related to this proposal, Geyer (7) reported that direct agonists of 5-HT_1 and 5-HT_2 receptors decrease locomotor activity while agonists of 5-HT_{1B} receptors activate locomotor activity. Therefore, different subsystems, maturing during development, may subserve varying aspects of the same behavior.

One behavior that is critical to a young rat's survival is the transport response (TR). Originally described by Brewster and Leon (3), the TR occurs between the ages of 8 and 28 days, a time when the rat is relatively active and relatively unwieldy. In response to being firmly grasped by its dorsal surface, the pup will actively flex and adduct its hindlimbs, extend and adduct its forelimbs, and adduct its tail, forming a compact package for transport and enabling the mother to more efficiently carry it. If the pup does not respond appropriately, the mother typically will drag, step on, and often abandon it.

Based upon previous reports (15,26,27), Wilson et al. (25) suggested that the TR is composed of two components, an initial quiescence followed by the active limb adduction. Interestingly, Brewster and Leon (3) reported that the TR could be elicited in 40-day-old animals, rats typically too old to show the response, if the pups had been handled daily between the ages of 20 and 40 days. They suggested that this effect was due to a suppression of defense reactions, thereby reducing behaviors that would interfere with the TR. Thus, behavioral suppression may be a key factor necessary for elicitation of the TR.

Given the aforementioned observations (3,12,14), it seemed reasonable that 5-HT systems might be involved in one or more components of the TR. In this article, we present the results of two experiments in which we administered 5-HT antagonists to 23-day-old rats (Experiment 1) or a 5-HT agonist to 30-, 40-, and 50-day-old rats (Experiment 2) and tested for TR. We hypothesized that if 5-HT systems are involved in elicitation of the TR, blocking those systems should suppress the response, while stimulating the systems perhaps would cause a reinstatement of the TR. In addition, to get a better understanding of the nature of these manipulations on TR, we also recorded the dorsal immobility response (DIR) durations, as DIR is a complex inhibitory behavior (21) that may be related to the TR (13,15).

GENERAL METHOD

Subjects

Subjects in these experiments were 160 (20 litters) Sprague–Dawley albino rats, 23, 30, 40, or 50 days of age at the time of testing. Rats were derived from an established breeding colony at Sam Houston State University, and were housed in Plexiglas breeding cages on a 12 L:12 D schedule, with lights on at 0700 h. Food and water were available ad lib. As no differences in TR intensities have been measured between the light and dark phases of the cycle (23), all testing occurred between 0930 and 1200 h (light phase).

Materials

Quipazine, ketanserin, and metergoline were purchased from Research Biochemicals, Inc., Natick, MA. The vehicle substance, ascorbate, was purchased from Sigma Chemical Co., St. Louis, MO. Cinanserin was generously donated by Bristol-Myers Squibb, Princeton, NJ. Doses for specific drugs were based upon prior research (4,11,17–19).

Procedure

Prior to parturition, pregnant female rats were placed in Plexiglas breeding cages containing wood chips as bedding. Cages were checked daily at 0900 and 1600 h for the presence of newborn litters. The day a litter was first detected was considered postpartum day (PPD) 0. On PPD 1, litters were culled to 8–10 animals, and on PPD 21, subjects were weaned but continued to be housed in groups in breeding cages. On the day of testing, pups were removed from their home cages, placed in breeding cages containing fresh bedding, and randomly assigned to various drug-dosage groups (n = 10), based upon a split-litter design. Because no sex differences are apparent in TR intensities (23), sex of the subject was not considered a factor.

Prior to testing, each rat was given the appropriate drug regimen, removed to a second room, and marked with a felt-tipped pen for later identification, and then tested as described below. Blind experiments were performed, as the experimenters handling the rats and the individuals scoring the responses were unaware of any particular treatment of any particular animal.

Following drug administration, each animal was subjected to two behavioral measures. All rats first were tested for TR intensity. This consisted of an experimenter grasping the pup by the nape of the neck between the experimenter's thumb and first two fingers and firmly squeezing [see (28)]. Response intensity was recorded on a scale of 0 to 5, with one point awarded for each forelimb, hindlimb, and/or tail that was adducted to the subject's ventrum. The rat was given three trials with approximately 2-min intertrial intervals.

DIR duration was then measured. This consisted of gently holding the pup by the skin of the nape of the neck between the experimenter's thumb and index finger and suspending it above a tabletop. Duration was measured from the time of suspension until the pup made an escape response, defined as an abrupt jerking of its body, directed at the experimenter's hand. Subjects were given three trials with intertrial intervals of approximately 5 min and a maximum of 300 s for each trial [see (13)].

Data Analysis

Data were analyzed using analyses of variance (ANOVAs) with Newman–Keuls a posteriori procedures being used to determine differences between specific groups. Differences with a probability of less than 0.05 were considered to be statistically significant.

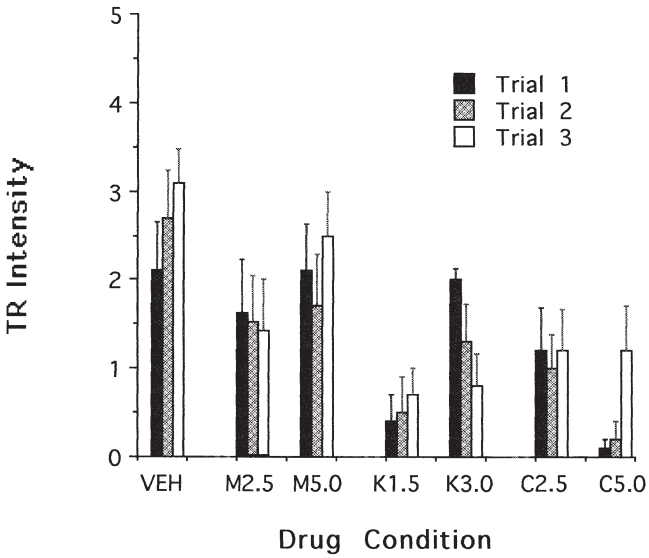

FIG. 1. TR intensity in 23-day-old rats as a function of dose of metergoline (M), ketanserin (K), or cinanserin (C), and trial.

EXPERIMENT 1

In this experiment, to assess if 5-HT systems are involved in the TR when the response occurs naturally, we administered varying doses of the 5-HT antagonists metergoline, ketanserin, and cinanserin to 23-day-old rats and tested for TR intensity and DIR duration. If one or more of the components of the TR is subserved by 5-HT systems, then blocking those systems should result in decreases in TR intensity.

Procedure

Thirty minutes prior to testing, 70 23-day-old rats were randomly assigned to seven drug conditions (n = 10) and

TABLE 1

MEAN TR INTENSITIES (+SE), COLLAPSED ACROSS TRIALS, AS A FUNCTION OF DRUG CONDITION

Condition		
Vehicle	2.63*	
	(0.42)	
Metergoline	2.5 mg/kg	5.0 mg/kg
	1.50	2.30†
	(0.54)	(0.44)
Ketanserin	1.5 mg/kg	3.0 mg/kg
	0.53	0.77
	(0.26)	(0.27)
Cinanserin	2.5 mg/kg	5.0 mg/kg
	1.13	0.50
	(0.37)	(0.19)

*Denotes different from all groups except the 5.0-metergoline group.

†Different from all groups except vehicle group.

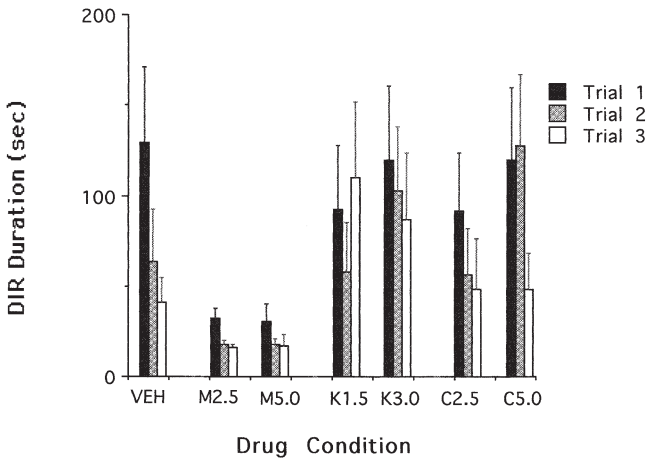

FIG. 2. DIR duration in 23-day-old rats as a function of dose of metergoline (M), ketanserin (K), or cinanserin (C) and trial.

given intraperitoneal (IP) administrations of either 0.2% ascorbate, metergoline (2.5 or 5.0 mg/kg), ketanserin (1.5 or 3.0 mg/kg), or cinanserin (2.5 or 5.0 mg/kg). The rats were then tested as described above.

Results

TR intensities for this experiment are presented in Fig. 1. To better see the drug effects reported here, these data, collapsed over trials, are presented in Table 1. A two-factor (drug condition × trials) ANOVA revealed a statistically significant drug effect, $F(6, 63) = 4.91$, $p < 0.05$, a significant trials effect, $F(2, 126) = 3.99$, $p < 0.05$, and a nonsignificant drug × trials interaction, $F(12, 126) = 1.35$, NS. Collapsed across trials, post hoc procedures revealed that rats in the 0.0-mg/kg group had stronger TR intensities than subjects in all other groups except for rats in the 5.0-mg/kg metergoline group. Rats in the 5.0-metergoline group had TRs more intense that rats in the 1.5- and 3.0-ketanserin and 2.5- and 5.0-cinanserin groups. With respect to the trials effect, TRs were more intense in trial 3 than in trial 1. No other differences were statistically significant.

DIR durations are presented in Fig. 2. These data, collapsed over trials, are presented in Table 2. An ANOVA on these data revealed a significant trials effect, $F(2, 126) = 5.44$, $p < 0.05$, with a nonsignificant drug effect, $F(6, 63) = 2.17$, NS, and a nonsignificant drug × trials interaction, $F(12, 126) = 1.31$, NS. Post hoc analyses showed that DIR durations were longer in trial 1 than in trials 2 or 3. No other differences were significant.

TABLE 2

MEAN DIR INTENSITIES (+SE), COLLAPSED ACROSS TRIALS, AS A FUNCTION OF DRUG CONDITION

Condition		
Vehicle	77.81 (25.60)	
Metergoline	2.5 mg/kg	5.0 mg/kg
	21.77 (2.43)	21.55 (5.29)
Ketanserin	1.5 mg/kg	3.0 mg/kg
	86.72 (28.13)	103.24 (32.25)
Cinanserin	2.5 mg/kg	5.0 mg/kg
	65.05 (25.07)	98.34 (23.78)

EXPERIMENT 2

Early-maturing 5-HT systems play an important role in facilitating age-appropriate behaviors, for example, suckling, with many of these behaviors being suppressed as these systems further develop (16). To determine if the TR is being suppressed, either by later-developing 5-HT components or by other neurotransmitter systems, we chose to attempt to reinstate the response by stimulating 5-HT systems in rats typically too old to show the response. If 5-HT systems are involved, then stimulating those systems should produce increments in response intensity.

Procedure

Subjects were 90 30-, 40-, and 50-day-old rats. Within age groups, each subject was randomly assigned to one of three dosage groups and given an IP administration of either 0.0, 2.5, or 5.0 mg/kg of quipazine. The subjects were then tested as described above.

Results

Data for TR intensity in this experiment are presented in Fig. 3. These data, collapsed over trials, are presented in Table 3. A three-way (age × dose × trial) ANOVA revealed a significant age effect, $F(2, 81) = 5.92$, $p < 0.05$, a significant dose effect, $F(2, 81) = 43.77$, $p < 0.05$, and a significant trials effect, $F(2, 162) = 22.69$, $p < 0.05$. The age × dose interaction was not statistically significant, $F(4, 81) = 1.28$, NS, the age × trials effect was not significant, $F(4, 162) = 0.61$, NS, the dose × trial effect was not significant, $F(4, 162) = 1.58$, NS, and the age × dose × trials triple interaction was not statistically significant, $F(8, 162) = 0.46$, NS. Post hoc analyses revealed that doses of 2.5 and 5.0 mg/kg of quipazine resulted in significant increases in TR intensity as compared with 0.0 mg/kg. Also, 30-day-old rats had significantly more intense TR intensities than had 40- or 50-day-old rats. In addition, TR intensity in trial 3 was significantly stronger than in trial 1. No other differences were statistically significant.

Data for the DIR are presented in Fig. 4. Mean drug effects, collapsed over trials, are presented in Table 4. An ANOVA revealed a significant dose effect, $F(2, 81) = 22.67$, $p < 0.05$, a significant trials effect, $F(2, 162) = 25.00$, $p < 0.05$, and a significant dose × trial interaction, $F(4, 162) = 2.76$, $p < 0.05$. The age effect was not significant, $F(2, 81) = 0.97$, NS, the age × dose interaction was not significant, $F(4, 81) = 1.50$, NS, and the age × trials effect was not significant, $F(4, 162) = 1.20$, NS. The age × dose × trials interaction was not statistically significant, $F(8, 162) = 0.81$, NS. Post hoc analyses revealed that in trials 1 and 2, DIR durations were greater for rats in the 5.0-mg/kg groups than in the 0.0- and 2.5-mg/kg groups. In trial 3, rats in the 5.0-mg/kg groups had durations greater than rats in the 0.0-mg/kg groups. No other differences were statistically significant.

GENERAL DISCUSSSION

Ristine and Spear (16) asserted that precocial portions of 5-HT systems mediate some behaviors critical to survival in young animals. During maturation, these early systems may be

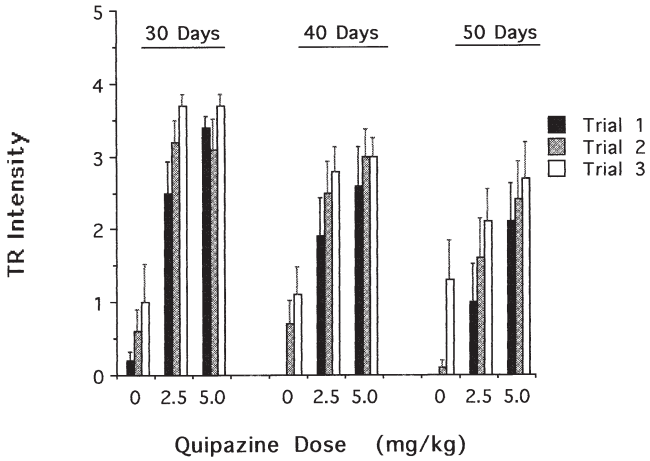

FIG. 3. TR intensity as a function of age, drug dosage, and trial.

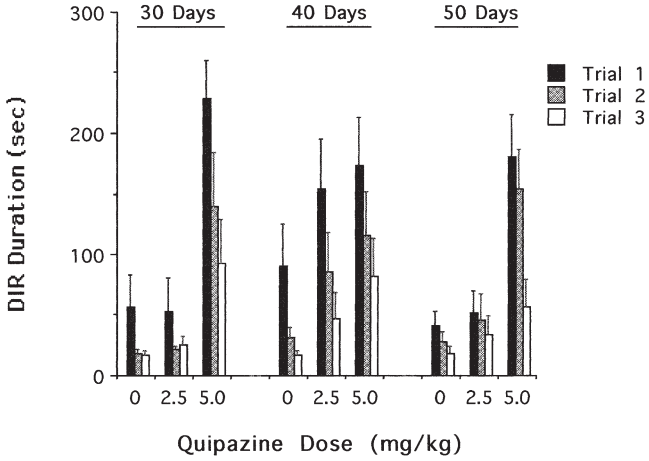

FIG. 4. DIR duration as a function of age, drug dosage, and trial.

suppressed by later-developing portions, which subserve adult-typical behaviors. In the experiments presented here, we tested the notion that 5-HT systems are involved in the TR, a behavior that is crucial to survival of young rats and is suppressed later on, as these animals reach adulthood. We hypothesized that if 5-HT systems are involved in the TR, then administering 5-HT antagonists should suppress the response in younger animals and administering a 5-HT agonist should reinstate the response in older animals. In both cases, these hypotheses were confirmed.

Data from Experiment 1 show that ketanserin and cinanserin, but not metergoline, were effective in eliminating the TR in young rats. There did appear to be a slight, albeit nonsignificant, decrease in TR intensity with 2.5 mg/kg of metergoline, but the high TR intensities in the 5.0-mg/kg group were very consistent. Therefore, we are reluctant to write these results off as a spurious finding. Perhaps the systems inducing the TR are not sensitive to metergoline in young animals or interference from other systems masked any effects of this particular drug. Regardless, two of the three antagonists suppressed the TR, indicating that 5-HT systems are functional with respect to the behavior in 23-day-old rats.

Data from Experiment 2 show a reinstatement of the TR with quipazine. One possible explanation for this effect is that the drug suppressed motor output, reducing behaviors which normally interfere with the TR, for example, struggling, and inducing quiescence, an initial component of the TR (25). A second possibility is that administration of the drug resulted in an increase in locomotor activity. Geyer (7) reported that the predominant effect of particular 5-HT releasers on subreceptor systems is to stimulate locomotor activity. If the effect we reported in this study was a function of locomotor activation, then we would assume that the quipazine administered here stimulated the second component of the TR, active limb adduction. The results of quipazine's effect on DIR duration in Experiment 2 lead us to believe that the former explanation, quipazine decreased motor output, is the more reasonable explanation at this time.

Several authors have proposed that the TR is related to DIR (13,15), and that appears to be the case with data from Experiment 2, reported here. Two caveats to this conclusion must be considered, though. First, 5-HT blockers failed to have a significant effect on DIR in Experiment 1. This lack of effect may be a function of age, a variable that has been shown to affect DIR's response to drugs (13), and later-developing portions of 5-HT systems may be more susceptible to chemical intervention, producing an effect on DIR in older but not younger rats. A second caveat is that we reported previously (25), and here, sensitization effects for both TR and DIR with repeated stimulation; TR increases and DIR decreases with repeated trials. The lack of a consistent positive linear relationship, both across trials and between ages, be-

TABLE 3

MEAN TR INTENSITIES (+SE), COLLAPSED ACROSS TRIALS, AS A FUNCTION OF AGE AND QUIPAZINE DOSE

	Dose		
Age	0.0 mg/kg	2.5 mg/kg	5.0 mg/kg
30 Days*	0.60	3.13†	3.40†
	(0.28)	(0.12)	(0.17)
40 Days	0.60	2.40†	2.87†
	(0.22)	(0.38)	(0.34)
50 Days	0.47	1.56†	2.40†
	(0.19)	(0.46)	(0.49)

*Denotes different from 40 and 50 days.
†Different from 0.0-mg/kg groups.

TABLE 4

MEAN DIR INTENSITIES (+SE), COLLAPSED ACROSS TRIALS, AS A FUNCTION OF AGE AND QUIPAZINE DOSE

	Dose		
Age	0.0 mg/kg	2.5 mg/kg	5.0 mg/kg*†
30 Days	30.34	31.16	153.92
	(8.94)	(9.21)	(26.93)
40 Days	46.43	95.70	123.56
	(12.23)	(26.57)	(28.21)
50 Days	28.95	43.73	130.45
	(7.71)	(14.52)	(22.11)

*Denotes different from 0.0- and 2.5-mg/kg groups in Trials 1 and 2.
†Different from 0.0-mg/kg groups in Trial 3.

tween TR and DIR is a cause of some concern. One possibility for this discrepancy is that different neurotransmitter systems may be subserving these sensitization effects and currently we are considering this prospect.

As has been suggested for other behaviors (16), the underlying neurochemical cause for the suppression of the TR in older animals may be late-developing 5-HT systems. If this is the case, giving quipazine, which has an affinity for 5-HT_3 receptors (8), may induce enough activity in these receptors to overcome more rostrally occurring systems and the normal behavioral suppression that occurs during ontogeny. The result is that the response returns.

An alternative explanation, though, is that quipazine may be working indirectly through other systems. Stimulation of 5-HT_3 receptors has been shown to inhibit acetylcholine release (1). Stimulation of these receptors also results in striatal dopamine release (2). Both of these systems, acetylcholine and dopamine, have been shown to be involved in elicitation and/or suppression and reinstatement of the TR (15,24–26). Therefore, overcoming the apparent suppression of the TR with 5-HT stimulation during adulthood may be through direct or indirect mechanisms. We currently are addressing that possibility.

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REFERENCES

1. Barnes, J. M.; Barnes, N. M.; Costall, B.; Naylor, R. J.; Tyers, M. B.: 5-HT_3 receptors mediate inhibition of acetyl choline release in cortical tissues. Nature 338:762–763; 1989.
2. Blandina, P.; Goldfarb, J.; Green, J. P.: Activation of a 5-HT_3 receptor releases dopamine from rat striatal slice. Eur. J. Pharmacol. 155:349–350; 1988.
3. Brewster, J.; Leon, M.: Facilitation of maternal transport by Norway rat pups. J. Comp. Physiol. Psychol. 94:80–88; 1980.
4. DeNoble, V. J.; DeNoble, K. F.; Spencer, K. R.: Protection against hypoxia-induced passive avoidance deficits: Interactions between DuP 996 and ketanserin. Brain Res. Bull. 26:817–820; 1991.
5. Elliott, P. J.; Walsh, D. M.; Close, S. P.; Higgins, G. A.; Hayes, A. G.: Behavioural effects of serotonin agonists and antagonists in the rat and marmoset. Neuropharmacology 29:949–956; 1990.
6. Fibiger, H.; Campbell, B. A.: The effect of parachlorophenylalanine on spontaneous locomotor activity in the rat. Neuropharmacology 10:25–32; 1971.
7. Geyer, M. A.: Serotonergic functions in arousal and motor activity. Behav. Brain Res. 73:31–35; 1996.
8. Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B.: The identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature 330:746–748; 1987.
9. Lucot, J. B.; Seiden, L. S.: Effects of serotonergic agonists and antagonists on the locomotor activity of neonatal rats. Pharmacol. Biochem. Behav. 24:537–541; 1986.
10. Mabry, P. D.; Campbell, B. A.: Serotonergic inhibition of catecholamine-induced behavioral arousal. Brain Res. 49:381–391; 1973.
11. Mazzola-Pomietto, P.; Aulakh, C. S.; Tolliver, T.; Murphy, D.: Functional subsensitivity of 5-HT-sub2A and 5-HT-sub2C receptors mediating hyperthermia following acute and chronic treatment with 5-HT-sub2a/sub2c receptor antagonists. Psychopharmacology (Berlin) 130:144–151; 1997.
12. McEwen, M. L.; Van Hartesveldt, C.; Stehouwer, D. J.: L-DOPA and quipazine elicit air-stepping in neonatal rats with spinal cord transections. Behav. Neurosci. 111:825–833; 1997.
13. Meyer, M. E.; Smith, R. L.; Van Hartesveldt, C.: Haloperidol differentially potentiates tonic immobility, the dorsal immobility response, and catalepsy in the developing rat. Dev. Psychobiol. 17:383–389; 1984.
14. Nanry, K. P.; Tilson, W. A.: The role of 5-HT_{1A} receptors in the modulation of the acoustic startle reflex in rats. Psychopharmacology (Berlin) 97:507–513; 1989.
15. Potter, T. J.; Cottrell, G. A.; Van Hartesveldt, C.: Effects of cholinergic agonists on the dorsal immobility response. Pharmacol. Biochem. Behav. 36:77–80; 1990.
16. Ristine, L. A.; Spear, L. P.: Is there a "serotonergic syndrome" in neonatal rat pups? Pharmacol. Biochem. Behav. 22:265–269; 1985.
17. Routsalainen, S.; Sirvio, J.; Jakala, P.; MacDonald, E.; Riekkinen, P., Sr.: Differential effects of three 5-HT receptor antagonists on the performance of rats in attentional and working memory tasks. Eur. Neuropsychopharmacol. 7:99–108; 1997.
18. Smith, R. L.; Barrett, R. J.; Sanders-Bush, E.: Neurochemical and behavioral evidence that quipazine-ketanserin discrimination is mediated by $serotonin_{2a}$ receptor. J. Pharmacol. Exp. Ther. 275:1050–1057; 1995.
19. Spear, L. P.; Ristine, L. A.: Quipazine-induced behavior in neonatal rat pups. Pharmacol. Biochem. Behav. 14:831–834; 1981.
20. Spear, L. P.; Ristine, L. A.: Suckling behavior in neonatal rats: Psychopharmacological investigations. J. Comp. Physiol. Psychol. 96:244–255; 1982.
21. Webster, D. G.; Lanthorn, T. H.; Dewsbury, D. A.; Meyer, M. E.: Tonic immobility and dorsal immobility in twelve species of muroid rodents. Behav. Neural. Biol. 31:32–41; 1981.
22. Williams, C.; Rosenblatt, J. S.; Hall, W. G.: Inhibition of suckling in weaning-age rats: A possible serotonergic mechanism. J. Comp. Physiol. Psychol. 93:414–429; 1979.
23. Wilson, C.: unpublished observations.
24. Wilson, C.: The effects of apomorphine and isoproterenol on the "transport response" in white rats. Int. J. Dev. Neurosci. 3:279–284; 1985.
25. Wilson, C.; Brocher, N.; Copeland, A.; Thornton, H.; Godfrey, J.: Effects of atropine and adrenergic antagonists on arousal in rats. J. Gen. Psychol. (in press).
26. Wilson, C.; Cromey, A.: Evidence of a cholinergic input into the suppression of the transport response in white rats. Psychobiology 17:43–48; 1989.
27. Wilson, C.; Cromey, A.: The effects of varying doses of pilocarpine on the transport response in white rats. Bull. Psychonom. Soc. 27:138–140; 1989.
28. Wilson, C.; Cromey, A.; Kramer, E.: Tactile, maternal, and pharmacologic factors involved in the "transport response" in rat pups. Anim. Learn. Behav. 17:373–380; 1989.