Pharmacology Biochemistry and Behavior, Vol. 43, pp. 1273–1277, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Serotonin Receptor Ontogeny: Effects of Agonists in 1-Day-Old Rats

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Received 19 June 1992

PRANZATELLI, M. R. *Serotonin receptor ontogeny: Effects of selective agonists in 1-day-old rats.* PHARMACOL BIOCHEM BEHAV **43**(4) 1273–1277, 1992.—Although numerous subtypes of serotonin [5-hydroxytryptamine (5-HT)] receptors have been identified in the newborn rat by radioligand binding studies, there have been few studies of the functional significance of these early receptors, most without the benefit of selective drugs. We performed acute dose–response and time course behavioral studies in 1-day-old rats with the putative selective agonists 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (5-HT_{1A}), 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1*H*-indole (RU 24969) (5-HT_{1B}), and (±)1-(2,5-dimethoxy-4-iodo-phenyl aminopropane)-2 (DOI) (5-$HT_{2/1C}$). The agonists induced distinctive behavioral syndromes. The DOI syndrome mainly included rudiments of forepaw myoclonus and dystonic limb postures, but no shaking behavior (head shakes or wet-dog shakes) or spinal myoclonus, two key reference behaviors for its effects in adult rats. The most distinctive feature of the 8-OH-DPAT-induced syndrome was flat body posture. RU 24969 most significantly increased locomotor activity, inducing propulsive movements with episodic rests and sudden hindlimb jerks. These studies suggest that functional and differential activity of 5-HT_{1A}, 5-HT_{1B}, and 5-$HT_{2/1C}$ receptors occurs much earlier in the rat than previously appreciated. The absence of DOI-induced shaking behavior and spinal myoclonus, however, suggests incomplete maturation at the level of the receptor or effector pathways for these behaviors.

Ontogeny Serotonin Receptor 8-OH-DPAT RU 24969 DOI

MULTIPLE subtypes of serotonin [5-hydroxytryptamine (5-HT)] receptors have been identified by radioligand binding studies in the mammalian CNS. 5-HT_1 and 5-HT_2 receptor recognition sites are present at birth but increase or decrease during postnatal development depending upon brain region and other factors (4,23,25). There is little information on whether these early receptors are functional.

In adult rats, activation of 5-HT_{1A}, 5-HT_{1B}, and 5-$HT_{2/1C}$ receptors has distinct behavioral correlates (6,8,21). Stimulation of 5-HT_{1B} sites has been associated with hyperlocomotion. Forepaw tapping and flat body posture, two behaviors of a complex syndrome of motor abnormalities described as the "serotonin syndrome," are evoked by activation of 5-HT_{1A} sites (6,21). 5-$HT_{2/1C}$ sites may apparently also participate in forepaw tapping, as well as in 5-HT-mediated shaking behavior (6,16) and spinal myoclonus (5,14). In developing rats, age-dependent differences in the appearance of the serotonin syndrome have been described (8,17), but few studies have used selective ligands, and none in 1-day-old rats. In rapid maturation of the rat brain, several days may make a difference.

To study the ontogeny and functional significance of 5-HT receptors, rat pups were injected with 5-HT agonists and behavioral effects were observed. We chose the putative agonists 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H-indole (RU 24969), 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), and 1-(2,5-dimethoxy-4-iodo-phenyl aminopropane)-2 (DOI) for selectivity at 5-HT_{1B}, 5-HT_{1A}, and 5-HT_2 receptors, respectively. We focused on behaviors that have been well characterized in adult rats.

METHOD

Animals and Drugs

Pregnant rats of 13 days gestation were obtained from Zivic-Miller (Pittsburgh, PA) and housed separately. A constant temperature (23°C) and 12 L : 12 D cycle were maintained.

These data were presented at the Annual Child Neurology Society Meeting, 1988.

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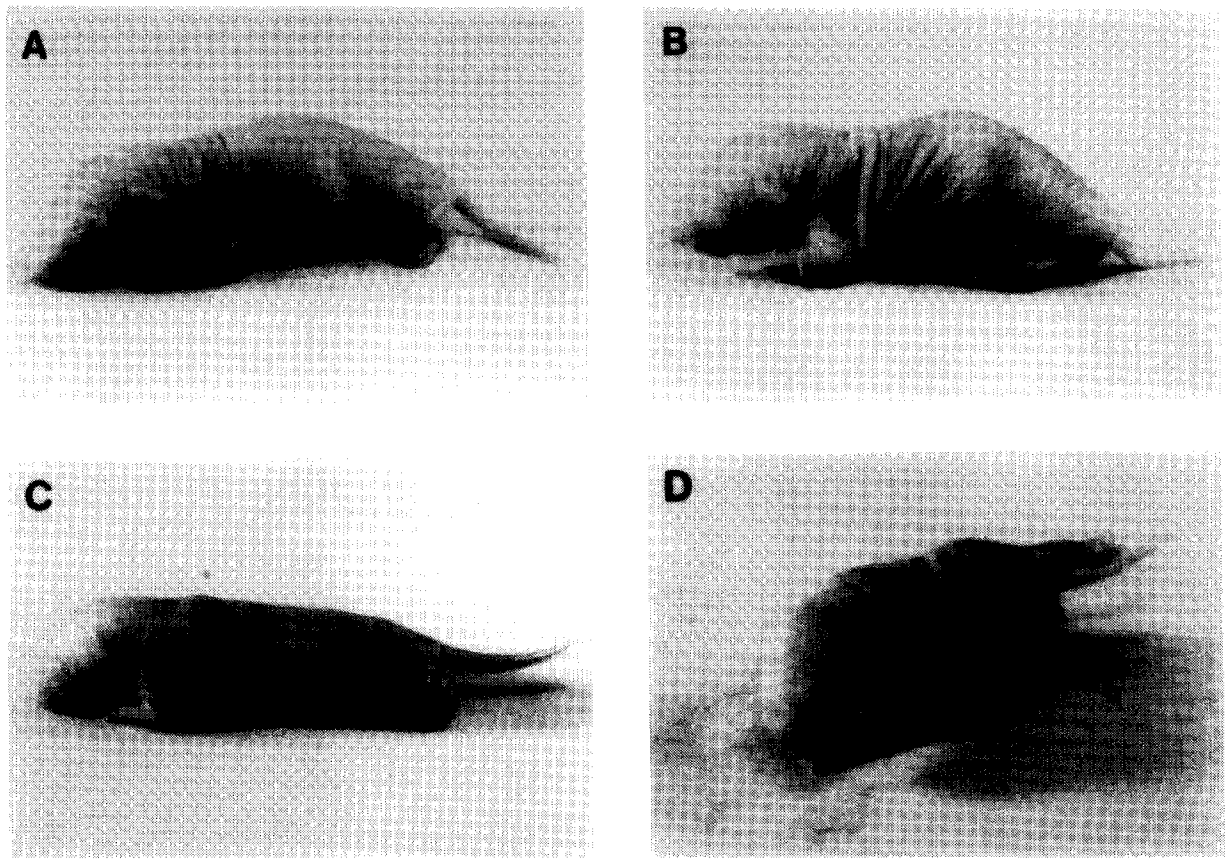

FIG. 1. Behavioral effect of acute IP injection of 3 mg/kg (A) saline, (B) 1-(2,5-dimethoxy-4-iodo-phenyl aminopropane)-2 (DOI), (C) 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), or (D) 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1*H*-indole (RU 24969) in 1-day-old rats. Several photographs were taken during peak behavioral drug effects and a representative one is shown. Behavioral differences between drugs were statistically significant ($p = 0.0001$) for forepaw tapping, $F(2,49) = 15.72$, hunching or arching of back, $F(2,49) = 18.91$, tail stiffening, $F(2,49) = 7.29$, mobility, $F(2,49) = 15.95$, and limb extension dystonic posture, $F(2,49) = 12.13$, ANOVA.

TABLE 1

5-HT AGONIST-EVOKED 5-HT-SYNDROME BEHAVIORS IN ONE DAY OLD RATS

		DOI (mg/kg)					
	Saline	0.05	0.1	0.2	1	2	3
FOR	1.6 ± 0.3	0.8 ± 0.5	3 ± 0	4.0 ± 1.2	11.0 ± 2.0*	12.3 ± 1.5*	11.7 ± 1.2*
HUN	3.9 ± 0.6	5.8 ± 0.3	6 ± 0	5.7 ± 0.3	11.3 ± 0.7*	5.7 ± 0.9	6.3 ± 0.3
SPR	2.7 ± 0.8	1.0 ± 0	0.7 ± 0.7	1.0 ± 0	0	1.3 ± 1.3	2.3 ± 1.2
STF	0.3 ± 0.1	0	0	0	0	1.7 ± 0.9	0
MOB	3.5 ± 1.0	4.5 ± 0.7	8.0 ± 1.5	5.3 ± 0.3	13.7 ± 1.3*	2.7 ± 0.7	12.7 ± 2.8*
LIMB	0.1 ± 0.1	0	0.3 ± 0.3	0	8.3 ± 2.3*	2.3 ± 0.9	2.7 ± 0.9

Data are means ± SEM ($n = 3$ per dose, 15 for saline). Behaviors were defined and scored as described in the Method section with a maximum score of 24. FOR, forepaw tapping; HUN, hunched or arched back; SPR, sprawling of limbs; STF, stiff or elevated tail; MOB, mobility; LIMB, limb extension dystonic posture. Dose effects were significant ($p = 0.0001$) for forepaw tapping, $F(6, 49) = 16.21$, hunching, $F(6, 49) = 5.63$, tail stiffening or elevation, $F(6, 49) = 5.84$, mobility, $F(6, 49) = 31.11$, and dystonic limb posture, $F(6, 49) = 10.58$, ANOVA. For sprawling, the effect of dose was weak, $F(6, 49) = 2.96$, $p = 0.025$.

*$p = 0.05$ compared to saline, SNK test.

Drugs were freshly prepared for each experiment and dosages were calculated according to the body weight determined on the day of testing. The volume of drug or vehicle was 1 ml/kg. RU 24969 (Roussel UCLAF, Paris, France), 8-OH-DPAT HBr [Research Biochemicals Inc. (RBI), Natick, MA], and ± DOI HCl (RBI) were dissolved in 0.9% saline.

Behavioral Scoring

Dams with their newborn pups were brought to the laboratory to acclimatize. A 21 × 24 × 45-cm clear plastic cage without bedding was partitioned into areas approximately three lengths of a newborn rat. Rat pups were taken from dams and injected intraperitoneally immediately before placement in the test cage with 0.9% saline or various doses of DOI, 8-OH-DPAT, or RU 24969. Pups received only a single drug at a single dose. Behaviors of the serotonergic syndrome were scored individually by a trained observer blinded to drug treatment on a five-point scale: absent [0], trace [1], mild or infrequent [2], moderate or intermittent [3], and severe or continuous [4] (16). The observations were scored for several seconds at 5-min intervals for 30 min after drug injection. This time was chosen because it allowed the maximum behavioral abnormalities to appear. Twelve scored behaviors included forepaw movements (forepaw treading, excessive placement, or tapping), hunching (back arching), sprawling (of limbs), backing, pivoting (tight circling), stiff tail, mobility (locomotion, including paddling), wet-dog shakes (including head shakes), spinal myoclonus (skin jerks or back muscle contractions), dystonic limb extension, body jerks, and lying on side. The observer also noted presence of head tremor, head weaving (lateral side-to-side repetitive movements), head nodding, flat body posture, barrier climbing, lying on sides, and any other observations. Several photographs were taken after drug or saline injection.

Statistics

We used analysis of variance (ANOVA) to test statistical effects of the independent variables drug and dose and the interaction of drug and dose on the various dependent behavioral variables. The sums of each 5-min score were taken for each pup and the data are means ± SEM of those sums. The statistical analysis was run using the SAS (PROC GLM), a computer software package (19). Only significant main effects ($p < 0.05$) were then analyzed by individual drugs using the Newman-Keuls multiple-range test of the means.

RESULTS

5-HT agonists induced distinctive motor syndromes even in newborn rats (Fig. 1). Statistical overall main effects were highly significant ($p = 0.0001$) for forepaw tapping, hunching, stiff tail, sprawling, limb extension dystonic posture, and mobility, $F(17, 49) = 15.72$, 18.91, 4.95, 7.29, 15.95, and 12.13, respectively, ANOVA. For each behavior, drug effects were dose dependent or dose related (Table 1).

None of the agonists studied evoked shaking behavior or spinal myoclonic jerks in the newborn rat. Spontaneous shaking behavior also was not observed. It would appear that drug-induced serotonergic behaviors override the expression of spontaneous neonatal myoclonic jerks only at the highest doses. Mean values of zero were also obtained for backing and pivoting, which were then dropped from further analysis.

Saline

Even saline-injected pups were often found on their sides and spontaneous motor activity included myoclonic jerks during presumed sleep and tremulousness and ataxia with locomotion. Oscillatory side-to-side head tremor of a few seconds duration was observed infrequently.

8-OH-DPAT

8-OH-DPAT flattened the body posture. We did not score this behavior directly but scores for hunching dropped below the score given to saline-injected controls and were typically zero values. There was also a tendency to straighten and elevate the tail, $F(5, 12) = 5.19$, $p = 0.0009$. Pups were also sprawled, $F(5, 12) = 10.76$, $p = 0.0004$. Head tremors and nodding were reported at the lower dose range.

DOI

DOI induced a "balancing on the wrist or ankle" postural abnormality of the limbs, $F(5, 13) = 9.62$, $p = 0.0005$. This

TABLE 1

CONTINUED

8-OH-DPAT (mg/kg)						RU 24969 (mg/kg)				
0.05	0.1	0.2	0.5	1	3	0.05	0.1	0.2	0.5	3
0	3.3 ± 0.9	3.7 ± 1.5	0.7 ± 0.7	5.3 ± 1.7	5 ± 2.7	1.3 ± 0.3	0.3 ± 0.3	0.7 ± 0.3	1.7 ± 0.7	0
1.3 ± 0.3	0	0	0	0	0	6 ± 0	5 ± 1	6 ± 0	3 ± 0*	0*
2.3 ± 1.5	3 ± 1.2	1.7 ± 0.3	3 ± 1.7	4.7 ± 0.7	11.3 ± 0.3*	1.7 ± 0.9	4.7 ± 1.2	2.3 ± 0.3	0.3 ± 0.3*	0*
1.0 ± 1.0	0	0	1.3 ± 1.3	4.0 ± 0.6*	5.7 ± 1.8*	1 ± 1	0	0	0	0
1.0 ± 1.0	1.0 ± 1.0	0	0	2.3 ± 1.9	16.7 ± 1.3*	4.3 ± 0.3	3.7 ± 2.7	9.7 ± 1.7	12 ± 1.2*	23.3 ± 0.3*
0	0	0	0	0	0	1.0 ± 0.6	0	0.7 ± 0.5	0.3 ± 0.3	0

posture is dystonic. On close observation, there were small-amplitude rippling movements of the digits, which looked like the rudiments of reciprocal forepaw treading and were scored accordingly, $F(5, 13) = 19.89, p = 0.0001$. The back of DOI-injected pups was slightly hunched or arched compared to rats injected with saline or other drugs, $F(5, 13) = 21.06$, $p = 0.0001$.

RU 24969

RU 24969 induced marked forward locomotion, paddling, barrier climbing with occasional twisting of the torso, and falling over. This was reflected in the composite behavior mobility, $F(4, 10) = 32.3$, $p = 0.0001$. With increasing dose, scores fell below those in saline-injected rats for hunching, $F(4, 10) = 32.5$, $p = 0.0001$, and sprawling, $F(4, 10) = 7.11, p = 0.006$. Infrequent myoclonic jerks of the hindlimbs only were also observed.

The time course of 5-HT agonist-induced behaviors was similar, with maximum scores reached by 10–15 min after drug injection.

DISCUSSION

The main finding of our study is that putative selective 5-HT receptor subtype agonists induce distinctive behavioral syndromes in the newborn rat. These data suggest that functional and differential activity of 5-HT_{1A}, 5-HT_{1B}, and 5-$HT_{2/1C}$ receptors in the developing rat occur much earlier than previously appreciated. Jacobs (8), comparing the serotonin syndrome in adult rats using 5-MeO-DMT, concluded that it did not occur in young rats until about 21 days. Afterwill and Green (1) reported that by 21 days there was no difference between pups and adult rats in sensitivity to 5-MeO-DMT or quipazine. Ristine and Spear (17) found that agonists did not induce a 5-HT syndrome identical to adults until 14–17 days postnatally, but they hypothesized that neonatal forelimb paddling, upward flexed limbs, and raised paws were rudiments of adult rat hyperlocomotion, hindlimb abduction, and reciprocal forepaw treading, features of the serotonin syndrome. The 5-HT agonists available for these earlier studies were nonselective.

Behavioral effects of more selective 5-HT agonists in the rat were first reported simultaneously by our lab (12) and Kirstein and Spear (9) and confirmed and extended soon afterward by Jackson and Kitchen through the use of 5-HT antagonists (7). There are similarities and differences in results and interpretation between the studies. We concur with Kirstein and Spear, who used 4-day-old rats injected SC, that the "unusual positioning of the limbs" is characteristic of DOI in neonates, but Jackson and Kitchen concluded that 0.1–10 mg/kg DOI IP had no behavioral effects in 5-day-old rats. Jackson and Kitchen also found that RU 24969 greatly increased locomotion, while Kirstein and Spear obtained the same result with TFMPP. All three studies found that 8-OH-DPAT induced hyperlocomotion or behavioral activation and flat body posture. We did not observe the convincing head weaving with 8-OH-DPAT or RU 24969 reported by Jackson and Kitchen, but they used higher doses and defined head weaving as both lateral and vertical head movement. This behavior was not evaluated by Kirstein and Spear. Our definition was rather stringent, perhaps too much so, requiring that the head must repetitively move from side to side across the midline as seen in adult rats exclusive of vertical movements. Jackson and Kitchen did not find that 8-OH-DPAT evoked forepaw tapping, we found a trace amount, and the behavior was not evaluated by Kirstein and Spear. The movements we scored are the rudiments of the more substantial forepaw tapping in adult rats. There are a number of methodological differences between the studies, including the behaviors scored.

We now extend the previous reports by studying younger rats and investigating a newly reported 5-$HT_{2/1C}$ receptor-mediated behavior, which has been called skin jerks (14), back muscle contractions (5), or spinal myoclonus (16). Unlike in adult rats, DOI did not evoke spinal myoclonus or shaking behavior, two of its distinctive behaviors, in newborn rats although it did induce forepaw tapping. Each of these DOI-evoked behaviors in adult rats has a similar dose–response (14). It is possible that sudden episodes of flipping over in the neonatal rat are the rudiment of wet-dog shakes, but the sudden and prominent spontaneous myoclonic jerks of rat pups provide a sufficient explanation.

There were also other important age-related differences in the effects of 5-HT agonists. RU 24969 evoked hindlimb jerks only in neonates, and 8-OH-DPAT-evoked locomotor stimulation was more prominent in neonates, making its effects more similar to RU 24969 than it is in adults at the same dose (15,16).

The scoring of serotonergic behaviors in the 1-day-old rat is challenging because of the high incidence of spontaneous myoclonus, a motor feature of paradoxical sleep, which manifests in all body parts (13). One-day-old rat pups are often lying on their sides rather than upright, introducing a postural variable that does alter motor behavior, such as bringing out forepaw tapping. Wakefulness, an important motor determinant, may be difficult to gauge. For these and other reasons, we suggest that neonatal behaviors be videotaped for repeated evaluation.

Our behavioral data concur with the appearance of 5-HT receptors measured by radioligand binding assays and autoradiographically, which show that both 5-HT_1 and 5-HT_2 receptors are present at birth (3,4,22,25) but have a different regional distribution and density than in adults. In the cerebral cortex and hippocampus, 5-HT_1 and 5-HT_2 binding sites increase steadily from birth (8–50% of adult levels) until approximately 21 days (3,10,22,25). 5-HT_{1A} receptors are expressed transiently in the newborn cerebellum (4). 5-HT_{1C} binding sites in whole rat brain increase rather than decrease during this time (18). Second messenger and electrophysiological studies of early 5-HT systems also reveal a maturational process (2,11,20). It has been suggested that even fetal 5-HT_1 receptors are functional (24).

The difference in ontogeny of drug-induced shaking behavior and other behaviors suggests differences in maturation of 5-HT_2 and 5-HT_1 sites or effector pathways. Intriguing possibilities are that the maturation of serotonin syndrome behaviors, elicitable even at birth, derive from increasing 5-HT receptor density or from elimination of transiently expressed sites. Further studies are indicated to chose between these alternatives.

ACKNOWLEDGEMENTS

This work was supported by NIH Grant 1-K08-NS01158 from the NINDS (CIDA), a grant from the Myoclonus Research Fund, and Grant R-381-88 from the United Cerebral Palsy Education and Research Foundation and the William Randolph Hearst Foundation, and the Children's Research Institute. The author thanks Betsy Schaefer for typing the manuscript, Veronica Hlibcuk for technical assistance, and Laura Birch for computer data entry.

REFERENCES

1. Afterwill, C. K.; Green, A. R. Response of developing rats to L-tryptophan plus an MAOI-I. Monitoring changes in behavior, brain 5-HT and tryptophan. Neuropharmacology 19:325–335; 1980.
2. Bennett, D. S.; Giarman, N. J. Schedule of appearance of 5-hydroxytryptamine (serotonin) and associated enzymes in the developing rat brain. J. Neurochem. 12:911–918; 1965.
3. Bennett, J. P.; Snyder, S. H. Serotonin and lysergic acid diethylamide binding in rat brain membranes: Relationship to postsynaptic serotonin receptors. Mol. Pharmacol. 12:373–389; 1976.
4. Davel, G.; Verge, D.; Becerril, A.; Gozlan, H.; Spampinato, U.; Hamon, M. Transient expression of 5-HT_{1A} receptor binding sites in some areas of the rat CNS during postnatal development. Int. J. Dev. Neurosci 5:171–180; 1987.
5. Fone, K. C. F.; Johnson, J. V.; Bennett, G. W.; Marsden, C. A. Involvement of 5-HT_2 receptors in the behaviors produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG3509) to rats. Br. J. Pharmacol. 96:599–608; 1989.
6. Goodwin, G. M.; Green, A. R. A behavioral and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT_1 and 5-HT_2 receptors. Br. J. Pharmacol. 84:743–753; 1985.
7. Jackson, H. C.; Kitchen, I. Behavioural profiles of putative 5-hydroxytryptamine receptor agonists and antagonists in developing rats. Neuropharmacology 28:635–642; 1989.
8. Jacobs, B. L. An animal behavioral model for studying central serotonergic synapses. Life Sci. 19:777–786; 1976.
9. Kirstein, C. L.; Spear, L. P.; 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptor agonists induce differential behavioral responses in neonatal rat pups. Eur. J. Pharmacol. 150:339–345; 1988.
10. Murrin, L. C.; Gibbens, D. L.; Ferrer, J. R. Ontogeny of dopamine, serotonin and spirodecanone receptors in rat forebrain—an autoradiographic study. Dev. Brain. Res. 23:91–109; 1985.
11. Nelson, D. L.; Herbet, A.; Adrien, J.; Bockart, J.; Hamon, M. Serotonin-sensitive adenylate cyclase and [^{3}H]serotonin binding sites in the CNS of the rat—II. Respective regional and subcellular distribution and ontogenic developments. Biochem. Pharmacol. 29:2455–2463; 1980.
12. Pranzatelli, M. R. Ontogeny of serotonergic behaviors in the rat: Putative selective agonists. Ann. Neurol. [abstr.] 24:350; 1988.
13. Pranzatelli, M. R. Antimyoclonic effect of MK 801: A possible role for NMDA receptors in developmental myoclonus of the neonatal rat. Clin. Neuropharmacol. 13:329–338; 1990.
14. Pranzatelli, M. R. Evidence for involvement of 5-HT_2 and 5-HT_{1C} receptors in the behavioral effects of the 5-HT agonist 1-(7,5-dimethoxy-4-iodophenyl aminopropane)-2 (DOI). Neurosci. Lett. 115:74–80; 1990.
15. Pranzatelli, M. R.; Jappay, E.; Snodgrass, S. R. Effects of 5-HT receptor subtype-selective drugs on locomotor activity and motor habituation in the DHT adult rat model. Pharmacol. Biochem. Behav. 27:497–504; 1987.
16. Pranzatelli, M. R.; Pluchino, R. S. The relation of central 5-HT_{1A} and 5-HT_2 receptors: Low dose agonist-induced selective tolerance in the rat. Pharmacol. Biochem. Behav. 39:407–413; 1991.
17. Ristine, L. A.; Spear, L. P. Is there a "serotonergic syndrome" in neonatal rat pups? Pharmacol. Biochem. Behav. 22:265–269; 1985.
18. Roth, B. L.; Hamblin, M. W.; Ciaranello, R. D. Developmental regulation of 5-HT_2 and 5-HT_{1C} *m*RNA and receptor levels. Dev. Brain Res. 58:51–58; 1991.
19. SAS/STAT user's guide, release 6.03. Cary, NC: SAS Institute; 1988.
20. Segal, M. Developmental changes in serotonin actions in rat hippocampus. Dev. Brain. Res. 52:247–252; 1990.
21. Tricklebank, M. D.; Forber, C.; Fozard, J. R. The involvement of subtypes of the 5-HT receptor and of catecholamine systems in the behavioral response to 8-hydroxy-2-(di-*n*-propylamino) tetralin in the rat. Eur. J. Pharmacol. 106:271–282; 1984.
22. Uphouse, L. L.; Bondy, S. C. The maturation of cortical binding sites. Dev. Brain. Res. 1:415–417; 1981.
23. Uzbekov, M. G.; Murphy, S.; Rose, S. P. R. Ontogenesis of serotonin "receptors" in different regions of rat brain. Brain Res. 168:195–199; 1979.
24. Whitaker-Azmitia, P. M.; Lauder, J. M.; Shemmer, A.; Azmitia, E. C. Postnatal changes in serotonin$_1$ receptors following prenatal alterations in serotonin levels: Further evidence for functional fetal serotonin$_1$ receptors. Brain Res. 433:285–289; 1987.
25. Zilles, K.; Schleicher, A.; Glaser, T.; Traber, J.; Rath, M. The ontogenetic development of serotonin (5-HT_1) receptors in various cortical regions of the rat brain. Anat. Embryol. 172:255–264; 1985.