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# Serotonergic modulation of the rat pup ultrasonic isolation call: studies with $5HT_1$ and $5HT_2$ subtype-selective agonists and antagonists

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**Abstract.** A modulatory role for serotonin has been described for the development and expression of the ultrasonic call of infant rat pups during brief maternal separations. In previous studies, serotonin reuptake inhibitors selectively reduced the rate of calling following acute administration to 9–11-day-old pups and a serotonin neurotoxin (MDMA) systematically disrupted the development of ultrasonic vocalizations but not other measures of motor development. In the current studies, we extended our investigations to include drugs with purported receptor subtype selectivities. Consistent with previous reports, acute administration of $5HT_{1A}$ agonists buspirone and 8-OH-DPAT ((±)-8-hydroxy-2-(di-N-propylamino)tetralin) reduced the rate of calling at doses which did not affect motor activity or core body temperature. The rate reducing effects of buspirone persisted up to 1 but not 2 h after injection. Administration of purported $5HT_{1B}$ receptor agonists, CGS12066B (7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-a] quinoxaline) and TFMPP (1-[3-fluoromethyl)phenyl]-piperazine) increased the rate of calling depending on the specificity of the drug for the $5HT_{1B}$ receptor. *d,l*-Propranolol, a $5HT_1$ receptor antagonist, blocked the effects of both 8-OH-DPAT and TFMPP. m-CPP (1-(3-chlorophenyl)piperazine) and DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane), drugs with putative actions at $5HT_{1C}$ and $5HT_2$ receptor sites both decreased calling but differed according to their effects on motor activity. Ritanserin, a $5HT_2$ and $5HT_{1C}$ antagonist, produced a dose-related increase in call rate. A dose of ritanserin with no apparent intrinsic effects effectively antagonized DOI rate reducing effects but potentiated the rate reducing effects of m-CPP. These data extend previous studies demonstrating a role for serotonin in the expression of rat pup separation calls and further demonstrate that 5HT may increase or decrease calling depending on with receptor subtype is affected.

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**Key words:** Attachment behavior – Vocalization – Infant rats – Serotonin – Development – Anxiety

Ultrasonic vocalizations emitted by rat pups during brief separations from maternal care have proven to be a sensitive probe for a variety of environmental and pharmacological stimuli (Hofer and Shair 1987; Kehoe 1988; Hofer et al. 1989). This behavioral response to separation has a number of useful features for pharmacological analysis and apparent construct validity for anxiety-related psychopathologies. The likelihood of calling has a reliable developmental course, emerging soon after birth, peaking during the second week of postnatal life, and then decreasing at the time of eye opening when the development of alternate strategies may occur (Noirot 1966; Insel et al. 1988). Some investigators have described these vocalizations as "separation-distress calls" because they are associated with vulnerability and may be a potent stimulus for maternal retrieval (Noirot 1972; Smotherman el al. 1974). The rate of rat pup distress vocalizations is inversely related to ambient temperature (Insel et al. 1988), and is also reduced by the presence of littermates or familiar bedding odors (Hofer and Shair 1987).

Rat pup vocal behavior is selectively affected by several psychoactive drugs. To date, this model appears to be most successful at detecting anxiolytic properties for a broad spectrum of drug classes. For example, calling is reduced by low doses of benzodiazepines (Insel et al. 1986; Gardner and Budhram 1987), opiates (Kehoe and Blass 1986; Winslow and Insel 1991), serotonin reuptake inhibitors (Winslow and Insel 1990a), and antagonists at the N-methyl-D-aspartate-glycine receptor complex (Winslow et al. 1990). Calling is increased by drugs with purported "anxiogenic" properties such as pentylenetetrazol and the benzodiazepine inverse agonist FG-7142 (Insel et al. 1986).

In a previous study, we demonstrated that lesions of serotonin pathways with the neurotoxin MDMA altered development of USV. Although these results suggest that serotonin is involved in the physiological mediation of USV, the specific serotonergic sub-system mediating calling was not systematically studied. Previous studies of drugs acting at $5HT_{1A}$ and $5HT_{1B}$ receptor sites have demonstrated opposing effects on a variety of physiological and behavioral measures including thermoregulation (Wozniak et al. 1988, 1989), penile erection (Berendsen and Broekkamp 1987) and ejaculation (Renyi and Lewander 1989), and feeding behavior (Hutson et al. 1989). Biochemical studies have suggested that these receptor subtypes may be differentiated by pre-versus post-synaptic localization (Engel et al. 1986), although this model has recently been questioned (Hamon et al. 1990). The physiologic effects of endogenous serotonin may represent a balance of effects at several receptor subtypes. In the current studies, we examine the dose-effect functions, time-course, and agonist-antagonist interactions of representative ligands at $5HT_1$ and $5HT_2$ receptor subtypes.

## Materials and methods

*Subjects.* Offspring of Sprague Dawley breeders (Taconic Farms, Germantown, New York) were housed with both parents in polycarbonate cages (55 × 31 × 21 cm) until 9–11 days old. The vivarium temperature and lighting were constant at 24° C and 14–20 h light-dark cycle. Thirty minutes before testing, litters were removed as a group from their home cage and placed in a new cage with a small amount of home-cage bedding. Cages were transported to a lighted testing room adjacent to the vivarium, with an ambient temperature maintained at 24 ± 1° C. Unless otherwise specified each pup was used once.

*Behavioral testing.* Beginning 30 min after removal from their home-cage, each pup was placed in a polycarbonate recording chamber (46 × 29 cm) with a 5 × 5 cm grid drawn on the floor. A microphone (Bruel and Kjaer, model 4385, Copenhagen) with a parabolic reflector was suspended 10 cm over the cage floor to record pup ultrasonic vocalizations. Ultrasonic calls were transformed by a digital sound spectrum analysis system providing on-line the number of calls in each 2-min session (Burkholder et al. 1982; Insel et al. 1986). The number of grid cells entered by the pup was also collected during this period by visual scoring. At the end of a 2-min recording period, each pup was weighed and rectal temperature was measured (probe: YSI-K74367). Geotaxis, defined as latency to turn against a 30° inclined plane, was measured to assess motor coordination. Following collection of these baseline measures, pups were injected with vehicle or drug subcutaneously at the nape of the neck, then returned to littermates. Approximately 30 min later, pups were retested to assess drug effects. In time-course studies, pups were re-tested over a 4-h period. Pups which failed to emit at least 60 calls (< 10% of pups tested) in the pretest were excluded from further study.

*Drug administration.* All drugs were administered subcutaneously, 30 min prior to testing. Each pup was used only once. Drugs were

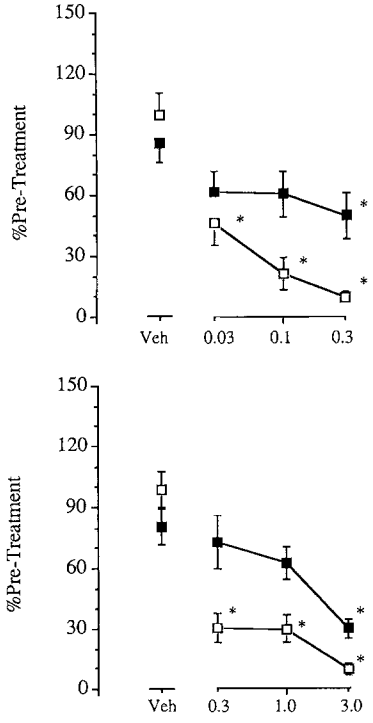


**Fig. 1.** The acute effects of buspirone *(top)* and 8-OH-DPAT *(bottom)* on the frequency of ultrasonic calling *(open symbols)* and grid cell crossing *(closed symbols)* during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test conducted immediately before drug injection. *Vertical lines* at each data point represent ± 1 SEM. *Asterisks* represent Dunnett *t*-comparisons ($P < 0.05$) in the presence of significant overall treatment effects detected by ANOVA

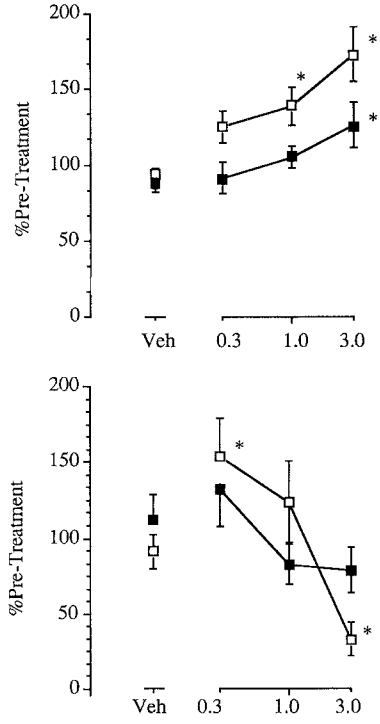


**Fig. 2.** The acute effects of CGS12066B *(top)*, and TFMPP *(bottom)* on the frequency of ultrasonic calling *(open symbols)* and grid cell crossing *(closed symbols)* during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test conducted immediately before drug injection. *Vertical lines* at each data point represent ± 1 SEM. *Asterisks* represent Dunnett *t*-comparisons ($P < 0.05$) in the presence of significant overall treatment effects detected by ANOVA

administered in a systematically varied order across litters (9–12 pups per litter, 9–11 days old, approximately 20 g body weight) typically with every dose of a particular drug represented in each litter. Five studies (using 34 litters) of the acute effects of drugs were conducted: (1) The dose effects of putative $5HT_{1A}$ agonists buspirone (0.3–3.0 mg/kg) and 8-OH-DPAT (0.03–0.3 mg/kg) were measured 30 min after treatment ($n=8$–10 per group); (2) The dose effects of putative $5HT_{1B}$ agonists TFMPP (0.3–3.0 mg/kg) and CGS12066B (0.3–3.0 mg/kg) were measured 30 min after treatment ($n=8$–10 per group); (3) A previous study of the acute effects of MDMA revealed a biphasic time-course with calling initially suppressed then "rebounding" to supra-normal levels (Winslow and Insel 1990b). To evaluate the possibility that a similar biphasic function may be associated with administration of $5HT_1$ receptor agonists, the time course of selected doses of buspirone (1.0 mg/kg), CGS12066B (1.0 mg/kg) and vehicle control was measured over a 4-h period using a within subject desing ($n=6$ per drug); (4) The effects of the non-selective $5HT_1$ antagonist *d,l*-propranolol (0.1–10.0 mg/kg) was measured alone or co-administered (5.0 mg/kg) with of TFMPP (0.5 mg/kg) or 8-OH-DPAT (0.5 mg/kg); (5) The acute effects of the $5HT_{1C}/5HT_2$ agonists DOI (0.03–0.3 mg/kg), and m-CPP (0.1–5.0 mg/kg) and the antagonist ritanserin (0.3–3.0 mg/kg) were measured alone or in combination.

*Drug preparation.* Buspirone (Sigma, St Louis, Mo.), 8-OH-DPAT, TFMPP, m-CPP, CGS12066B, *d,l*-propranolol, and DOI were dissolved in physiological saline. Ritanserin (Janssen Pharmaceutica, Beerse, Belgium) was initially dissolved in 20% methanol, then diluted in physiological saline immediately before injection. All concentrations were prepared to deliver drug in a volume of 0.1 ml/10 g body weight. Unless otherwise specified drugs were obtained from Research Biochemicals, Inc., Natick, Mass.

*Statistics.* Baseline differences and percent change from baseline for frequency of isolation calling, cell crossing, geotaxis and temperature were analyzed with multiple fixed-factor ANOVAs. Dunnett's *t*-tests were performed for comparison of acute drug effects against saline control, or for comparisons between all treatment means in the drug interaction studies (Winer 1971). For two-tailed distributions, $P<0.05$ was accepted as statistically significant.

## Results

### *Baseline measures*

No significant differences were detected for baseline measures of ultrasonic vocalizations, grid cell crossing, geotaxis or core body temperature within each drug treatment group. The mean and range for each drug group, collapsed across doses is portrayed in Tables 1 and 2 for reference.

**Table 1.** 5HT1 Agonists and antagonists

| Drug | Dose | *n*= | Vocalizations calls/2 min % Pre-treatment ±SEM | Activity cells/2 min % Pre-treatment ±SEM | Geotaxis latency (s) Δ Pre-treatment ±SEM | Body temperature °C Δ Pre-treatment ±SEM |
|---|---|---|---|---|---|---|
| 8-OH-DPAT | | | **(104.57± 7.86)** | **(73.45± 4.56)** | **(14.26±2.12)** | **(35.03±0.16)** |
| | 0.00 | 14 | 98.94± 8.79 | 80.45± 8.72 | −0.98±2.33 | 0.05±0.05 |
| | 0.30 | 7 | 30.15± 7.53* | 72.44±12.97 | −2.93±4.11 | 0.21±0.24 |
| | 1.00 | 8 | 29.59± 6.68* | 62.02± 7.93 | 3.21±1.90 | −0.67±0.42 |
| | 3.00 | 6 | 9.55± 3.21* | 29.81± 4.62* | 21.63±6.14* | −0.20±0.26 |
| Buspirone | | | **(104.75± 6.01)** | **(71.0± 4.28)** | **(11.85±1.44)** | **(34.85±0.14)** |
| | 0.00 | 6 | 99.47±10.95 | 85.79± 9.84 | −4.38±3.14 | −0.10±0.19 |
| | 0.03 | 6 | 46.15±11.35* | 61.41±10.06 | −2.78±2.93 | −0.10±0.25 |
| | 0.10 | 6 | 21.20± 7.94* | 60.48±11.18 | 1.59±3.67 | 0.10±0.37 |
| | 0.30 | 6 | 9.72± 2.72* | 49.88± 2.9* | 2.18±2.00 | −0.66±0.16 |
| CGS12066B | | | **(97.29± 6.49)** | **(73.0 ± 3.37)** | **(13.89±1.35)** | **(35.16±0.19)** |
| | 0.0 | 6 | 91.62± 5.24 | 87.13± 6.42 | −0.30±1.55 | −0.25±0.11 |
| | 0.3 | 6 | 123.32±10.68 | 89.28±10.60 | −4.52±2.51 | 0.17±0.21 |
| | 1.0 | 6 | 136.53±12.77* | 102.71− 7.29 | −5.16−2.53 | −0.25±0.17 |
| | 3.0 | 6 | 165.90±18.33* | 124.10±15.03* | −1.26±0.64 | −1.28±0.99 |
| TFMPP | | | **(107.56± 5.74)** | **(69.83± 3.84)** | **(12.55±1.76)** | **(35.38±0.16)** |
| | 0.0 | 14 | 91.35±11.03 | 112.37±16.34 | 1.49±1.70 | −0.30±0.23 |
| | 0.3 | 7 | 153.23±25.58* | 131.23±24.35 | −5.87±7.60 | −0.57±0.17 |
| | 1.0 | 7 | 123.31±27.16 | 82.30±13.20 | 6.34±8.40 | −0.57±0.23 |
| | 3.0 | 7 | 32.66±11.32* | 78.20±14.98 | −5.54±9.30 | −0.64±0.14 |
| d,l-Propranolol | | | **(110.54± 8.31)** | **(65.88± 2.12)** | **(11.80±1.49)** | **(35.17±0.15)** |
| | 0.0 | 6 | 105.62±14.07 | 77.29±12.25 | 2.38±3.57 | −0.51±0.57 |
| | 1.0 | 6 | 112.63±10.95 | 78.80±11.02 | −5.92±4.17 | −0.58±0.60 |
| | 5.0 | 6 | 111.72±15.93 | 66.63±11.91 | −2.68±1.84 | −0.17±0.21 |
| | 10.0 | 6 | 28.76± 7.34 | 67.54±11.58 | −0.36±3.69 | −0.83±0.46 |
| d,l-Propranolol | 5.0 | 6 | | | | |
| +DPAT | 0.3 | 6 | 61.82±10.12+ | 60.63±11.79 | −0.83±2.26 | −1.80±0.41 |
| +TFMPP | 0.5 | 6 | 51.42±10.92+ | 77.06± 7.97 | −3.96±3.30 | −0.80±0.34 |

Bold type in parentheses represents the mean±1 SEM for the pre-injection baseline at each measure. * Represents significant Dunnett's *t* comparison of drug effects with vehicle control ($P<0.05$), + represents comparisons between agonist and agonist-antagonist combinations in the presence of significant ANOVA

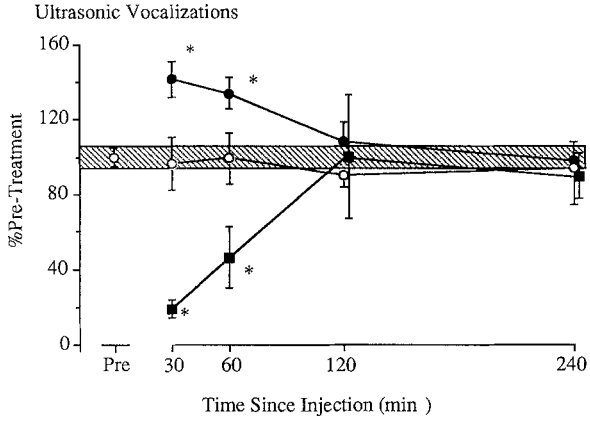


**Fig. 3.** The time course of 1.0 mg/kg buspirone *(closed squares)* and 1.0 mg/kg CGS12066B *(closed circles)* and saline *(open circles)* collected beginning 30 min after subcutaneous injection and measured in repeated 2-min tests at 60, 120, and 240 min after injection. Data are expressed as percent change from a 2-min pre-injection test conducted immediately before drug injection. *Vertical lines* at each data point represent ±1 SEM. The *horizontal bar* depicts ±1 SEM for a pre-treatment frequency of ultrasonic calling. *Asterisks* represent Dunnett *t*-comparisons ($P<0.05$) between vehicle and drug values at each time point in the presence of significant overall interaction effects detected by ANOVA between time since injection and treatment

## $5HT_1$

$5HT_{1A}$ receptor agonists buspirone [$F(3,23)=19.940$, $P<0.001$] and 8-OH-DPAT [$F(3,34)=24.638$, $P<0.001$] produced similar profound decreases in the rate of ultrasonic calling by separated rat pups (Fig. 1). Significant differences were detected at all three doses examined. Changes in the number of grid cells crossed were also detected but only after administration of the highest doses of both buspirone ($t_D=2.80$, $P<0.05$) and 8-OH-DPAT ($t_D=3.45$, $P<0.05$). Geotaxis was disrupted by the highest dose of 8-OH-DPAT ($t_D=4.59$, $P<0.05$) but not buspirone. Core body temperature was not significantly affected by either drug at the doses tested (Table 1).

The time course of buspirone's effects on ultrasonic calling (Fig. 3) revealed a significant reduction in calling beginning 30 min after injection ($t_D=5.06$, $P<0.05$) which continued up to 60 ($t_D=2.72$, $P<0.05$) but not 120 min. No evidence of a "rebound" effect was detected at 240 min after injection.

$5HT_{1B}$ agonists TFMPP and CGS12066B also significantly affected the rate of calling of rat pups; however, complex dose-response differences emerged (Fig. 2). CGS12066B produced a dose-related increase in calling [$F(3,20)=7.075$, $P<0.001$]. Increases were significant at both the 1.0 ($t_D=2.51$, $P<0.05$) and 3.0 mg/kg ($t_D=4.15$, $P<0.05$) doses. CGS12066B also increased the number of grid cell crossings exhibited at the 3.0 mg/kg ($t_D=2.51$, $P<0.05$). TFMPP increased the rate of calling, but only at the 0.3 mg/kg dose ($t_D=2.54$, $P<0.05$) while the highest dose significantly decreased calling ($t_D=2.41$, $P<0.05$). Core body temperature and geotaxis were not affected at the doses tested for either drug (Table 1). A study of the time course of CGS12066B effects on USVs revealed a significant increase in calling 30 min after injection ($t_D=3.16$, $P<0.05$) which remained high at 60 min ($t_D=2.79$, $P<0.05$) but returned to baseline at 120 min. As with buspirone no "rebound" effect was detected at 240 min (Fig. 3).

*d,l*-Propranolol (1.0–10.0) selectively affected the rate of ultrasonic calling in rat pups measured 30 min after injection [$F(3,23)=10.613$, $P<0.05$] but only at the highest dose. After 10.0 mg/kg *d,l*-propranolol, the rate of calling ($t_D=4.347$, $P<0.05$) was significantly reduced, while cell crossing, geotaxis, and core body temperature were unaffected at any of the doses tested (Fig. 4). Administration of a 5.0 mg/kg *d,l*-propranolol had no measurable effect on the behavior of pups but blocked the

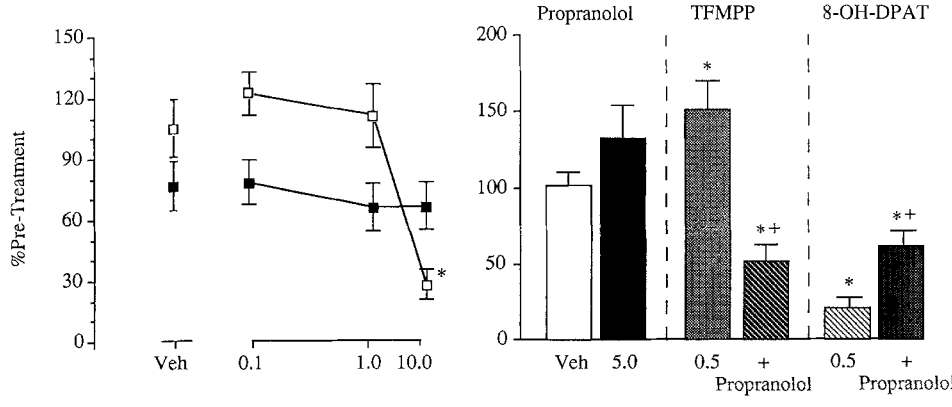


**Fig. 4.** The acute effects of *d,l*-propranolol *(left panel)* on the frequency of ultrasonic calling *(open symbols)* and grid cell crossing *(closed symbols)* during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test conducted immediately before drug injection. *Vertical lines* at each data point represent ±1 SEM. *Asterisks* represent Dunnett *t*-comparisons ($P<0.05$) in the presence of significant overall treatment effects detected by ANOVA. Also portrayed *(right panel)* are the effects of co-administration of *d,l*-propranolol (5.0 mg/kg) with TFMPP (0.5 mg/kg) or 8-OH-DPAT (0.5 mg/kg) on the frequency of ultrasonic calling. *Asterisks* represent Dunnett *t*-comparisons with vehicle control treatment ($P<0.05$), plusses represent Dunnett *t*-comparisons between agonist drug alone or in combination with *d,l*-propranolol, in the presence of significant overall treatment effects detected by ANOVA

rate reducing effects of 0.5 mg/kg 8-OH-DPAT ($t_D = 2.26$, $P < 0.05$) on calling compared to 8-OH-DPAT alone. Combined administration of 0.5 mg/kg TFMPP and 5.0 mg/kg *d,l*-propranolol was associated with a significant reduction in rate of calling compared to vehicle ($t_D = 3.261$, $P < 0.05$) and 0.5 mg/kg TFMPP alone ($t_D = 5.710$, $P < 0.05$) (Fig. 4).

### $5HT_{1C}/5HT_2$

m-CPP produced a dose related decrease in the rate of calling [$F(3,25) = 5.816$, $P < 0.001$] and increased grid cell crossings [$F(3,25) = 7.695$, $P < 0.001$] by infant rat pups. Calling was reduced at two doses of m-CPP (0.5: $t_D = 2.71$; 1.0: $t_D = 4.00$, $P < 0.05$). Grid cell crossing was increased at the highest dose (1.0: $t_D = 4.18$, $P < 0.05$). Acute administration of DOI significantly affected the rate of ultrasonic calling [$F(3,22) = 21.16$, $P < 0.05$] and grid cell crossing [$F(3,22) = 6.57$, $P < 0.05$] (Fig. 5). Calling was reduced at three doses of DOI (0.03: $t_D = 2.67$; 0.1: $t_D = 7.05$; 0.3: $t_D = 6.26$, $P < 0.05$). Grid cell crossing was reduced at the two higher doses (0.1: $t_D = 3.62$; 0.3: $t_D = 3.19$, $P < 0.05$).

The $5HT_{1C}/5HT_2$ receptor antagonist ritanserin produced a dose-related increase in the rate of calling [$F(3,27) = 13,69$, $P < 0.05$] at 1.0 ($t_D = 2.52$, $P < 0.05$) and 3.0 mg/kg ($t_D = 6.25$, $P < 0.05$) (Fig. 5). Locomotor activity, geotaxis and core body temperature were unaffected at the doses tested (Table 2). Combined administration of 0.1 mg/kg DOI and 0.3 mg/kg ritanserin resulted in a significant blockade of the reduction in calls ($t_D = 3.51$, $P < 0.05$) and grid cell crossing ($t_D = 2.11$, $P < 0.05$)

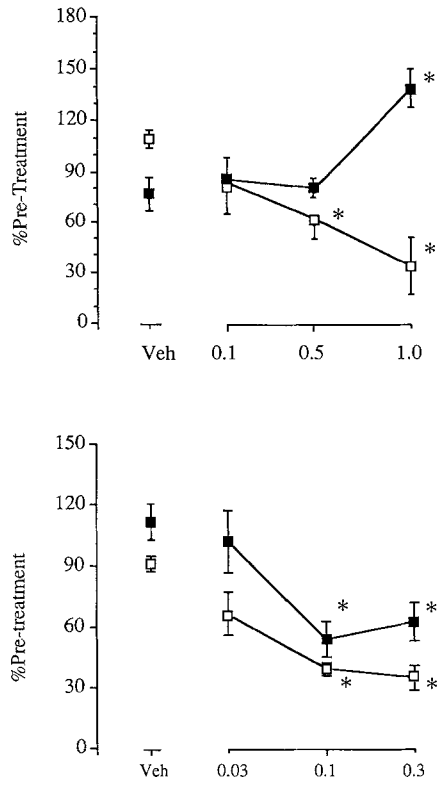


**Fig. 5.** The acute effects of m-CPP *(top)* and DOI *(bottom)* on the frequency of ultrasonic calling *(open symbols)* and grid cell crossing *(closed symbols)* during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test conducted immediately before drug injection. *Vertical lines* at each data point represent ± 1 SEM. *Asterisks* represent Dunnett *t*-comparisons ($P < 0.05$) in the presence of significant overall treatment effects detected by ANOVA

**Table 2.** $5HT_2/5HT_{1C}$ Agonists and antagonists

| Drug | Dose | *n* = | Vocalizations calls/2 min % Pre-treatment ± SEM | Activity cells/2 min % Pre-treatment ± SEM | Geotaxis latency (s) Δ Pre-treatment ± SEM | Body temperature °C Δ Pre-treatment ± SEM |
|---|---|---|---|---|---|---|
| m-CPP | | | **(112.11 ± 8.38)** | **(63.69 ± 2.97)** | **(9.14 ± 0.8)** | **(35.11 ± 0.23)** |
| | 0.00 | 7 | 110.98 ± 5.30 | 77.89 ± 10.29 | −1.61 ± 2.66 | −0.29 ± 0.31 |
| | 0.10 | 6 | 83.84 ± 18.68 | 85.28 ± 13.31 | −0.17 ± 2.03 | −0.42 ± 0.38 |
| | 0.50 | 7 | 61.24 ± 12.06* | 79.19 ± 5.64 | −0.23 ± 3.50 | −0.46 ± 0.12 |
| | 1.00 | 6 | 34.54 ± 16.61* | 138.19 ± 11.11* | 0.75 ± 2.99 | −0.67 ± 0.31 |
| DOI | | | **(119.57 ± 8.03)** | **(63.74 ± 3.67)** | **(6.74 ± 0.75)** | **(35.08 ± 0.12)** |
| | 0.00 | 6 | 91.29 ± 2.33 | 111.19 ± 8.88 | 1.67 ± 2.72 | −0.08 ± 0.15 |
| | 0.03 | 6 | 67.51 ± 10.49 | 103.42 ± 15.57 | 0.80 ± 1.63 | −0.20 ± 0.41 |
| | 0.10 | 6 | 33.59 ± 4.77* | 61.40 ± 7.81* | 0.86 ± 2.09 | −0.36 ± 0.14 |
| | 0.30 | 6 | 35.50 ± 6.30* | 63.39 ± 9.51* | 7.00 ± 2.63* | −0.80 ± 0.34 |
| Ritanserin | | | **(106.25 ± 7.48)** | **(41.64 ± 2.96)** | **(11.36 ± 1.23)** | **(34.27 ± 0.19)** |
| | 0.0 | 8 | 91.33 ± 9.58 | 88.00 ± 10.25 | 0.88 ± 2.11 | −0.06 ± 0.22 |
| | 0.3 | 6 | 117.22 ± 10.88 | 107.53 ± 14.36 | 4.40 ± 1.63 | −0.20 ± 0.12 |
| | 1.0 | 6 | 130.77 ± 12.52* | 84.01 − 16.91 | 3.33 ± 2.36 | −0.58 ± 0.35 |
| | 3.0 | 8 | 179.44 ± 10.51* | 87.58 ± 10.05 | 1.40 ± 2.85 | −0.06 ± 0.26 |
| Ritanserin | 0.3 | | | | | |
| +mCPP | 0.5 | 6 | 6.53 ± 0.793+ | 99.11 ± 16.61 | 2.83 ± 3.24 | 0.00 ± 0.00 |
| +DOI | 0.1 | 6 | 75.56 ± 4.823+ | 92.91 ± 7.98 | 0.33 ± 1.36 | 0.17 ± 0.21 |

Bold type in parentheses the mean ± 1 SEM for the pre-injection baseline at each measure. * Represents significant Dunnett's *t* comparison of drug effects with vehicle control ($P < 0.05$), + represents comparisons between agonist and agonist-antagonist combinations in the presence of significant ANOVA

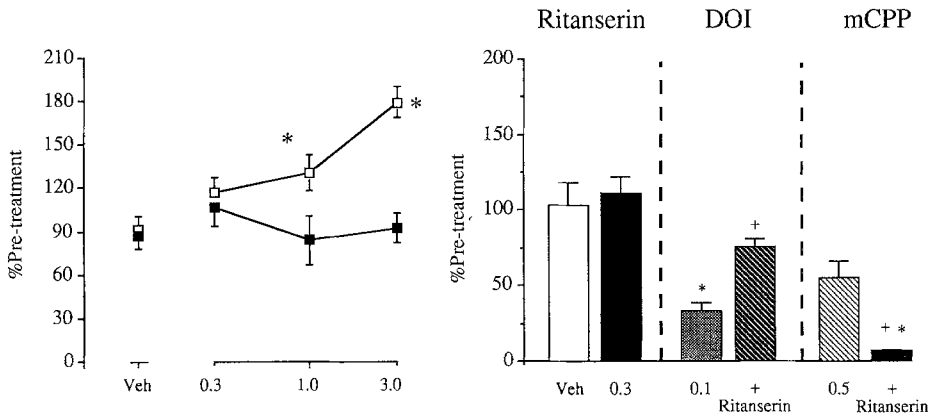


**Fig. 6.** The acute effects of ritanserin *(left panel)* on the frequency of ultrasonic calling *(open symbols)* and grid cell crossing *(closed symbols)* during a 2-min separation beginning 30 min after subcutaneous injection. Also presented *(right panel)* are the effects of co-administration of ritanserin (0.3 mg/kg) and DOI (0.1 mg/kg) or m-CPP (0.5 mg/kg) on the frequency of ultrasonic calling. *Asterisks* represent Dunnett's *t*-test comparisons of drug with vehicle control treatment ($P<0.05$), *pluses* represent Dunnett *t*-comparisons between DOI or m-CPP alone or in combination with ritanserin, in the presence of significant overall treatment effects detected by ANOVA

produced by DOI alone (Fig. 6). Combined administration of 0.5 mg/kg m-CPP and 0.3 mg/kg ritanserin did not block and instead appeared to potentiate the rate reducing effects of m-CPP on ultrasonic calling compared to m-CPP injected alone ($t_D=3.51$, $P<0.05$).

## Discussion

Serotonin receptor ligands selectively increase or decrease the rate of ultrasonic calling by infant rat pups depending on the receptor subtype affected. $5HT_{1A}$ and $5HT_2/5HT_{1C}$ agonists reliably decreased calling at relatively low doses. In contrast, both $5HT_{1B}$ agonists and a $5HT_2/5HT_{1C}$ antagonist increased calling at doses which did not appear to affect other measures of arousal. An extensive literature associates serotonin with the modulation of affective states (Soubrie 1986) and the development of psychopathology (see for example Erikkson and Humble 1990). Although in clinical studies, buspirone, an agonist at the $5HT_{1A}$ receptor site, is anxiolytic (Taylor 1987) and several serotonin reuptake inhibitors appear to be anti-obsessional (Insel and Winslow 1990), animal models developed for detecting the anxiolytic effects of benzodiazepines have not consistently shown efficacy for serotonergic agents (Broekemp and Jenk 1989; Insel and Winslow 1991).

The rat pup separation call can be reduced by several classes of anxiolytic drugs including the benzodiazepine agonists (Gardner 1985; Insel et al. 1986), glycine antagonists (Winslow et al. 1990), and the 5–HT reuptake inhibitors (Winslow and Insel 1990a). In addition, several putative anxiogenic compounds, such as pentylenetetrazol and FG–7142, increase the rate of calling (Insel et al. 1988). The rate-reducing effects of buspirone and 8-OH-DPAT reported here provide further evidence of the sensitivity and selectivity of the rat pup separation paradigm for the detection of anxiolytic efficacy. These findings replicate and extend previous reports (Hård and Engel 1988; Mos and Olivier 1989; Winslow and Insel 1990b) by demonstrating that drug induced decreases in calling recover within 2 h after injection and can be antagonized by the $5HT_1$ receptor antagonist *d,l*-propranolol.

The effects of $5HT_{1B}$ agonists CGS12066B and TFMPP varied according to the selectivity of each drug for the $5HT_{1B}$ receptor subtype. CGS12066B produced a reliable, dose-related increase in the rate of USV, while TFMPP also increased calling but only at low doses (0.3, 0.5 mg/kg) and decreased calling at a higher dose (3.0 mg/kg). Although both drugs have been characterized as preferential $5HT_{1B}$ agonists (Middlemiss and Hutson 1990), only CGS12066B has been characterized as selective, while TFMPP has significant activity at $5HT_{1C}$ and $5HT_2$ receptors (Neale et al. 1987; Titeler et al. 1987; Curzon and Kennett 1990; Middlemiss and Hutson 1990). While an increase in USV production has been noted with clinically anxiogenic compounds such as PTZ and FG–7142, neither TFMPP or CGS12066B has been tested clinically. In elevated plus-maze and social interaction tests, TFMPP has previously been characterized as "anxiogenic", but this action has been ascribed to stimulation of both $5HT_{1C}$ (Kennett et al. 1989) and $5HT_{1B}$ (Benjamin et al. 1990) receptors. Benjamin et al. (1990) failed to detect anxiogenic effects for CGS12066B and reported that CGS12066B blocked the anxiogenic effects of TFMPP. The current results in the rat pup suggest that high doses of TFMPP resemble m-CPP and DOI thus may reflect actions on $5HT_2/5HT_{1C}$ receptors (Kennett et al. 1989; Curzon and Kennett 1990; Hartig et al. 1990). This interpretation is supported by our finding that co-administration of a non-effective dose of *d,l*-propranolol with an excitatory dose of TFMPP resulted in a significant reduction of ultrasonic calling compared to increased calling produced by TFMPP alone. *d,l*-Propranolol at the dose used here preferentially antagonizes $5HT_{1A}$ and $5HT_{1B}$ receptors relative to $5HT_{1C}$ and $5HT_2$ receptors (Middlemiss and Hutson

1990). The current findings are consistent with the hypothesis that *d,l*-propranolol blocked the $5HT_{1B}$ mediated rate increasing effects of TFMPP which emerged at low doses, while not affecting or enhancing the $5HT_2/5HT_{1C}$ mediated rate decreasing effects which emerged at higher doses.

The $5HT_2/5HT_{1C}$ receptor agonists DOI (Shannon et al. 1984; Titeler et al. 1988) and m-CPP (Hamik and Peroutka 1989) produced a dose-related decrease in the rate of calling. Although these effects might suggest anxiolytic efficacy, both drugs had effects on associated behaviors: DOI significantly reduced motor activity and disrupted geotaxis whereas m-CPP increased motor activity. These motor disturbances suggest that changes in vocal behavior were non-specific. Curiously, the $5HT_2/5HT_{1C}$ receptor antagonist ritanserin increased the frequency of vocalization resembling an anxiogenic profile. The effects of m-CPP and ritanserin on pup behavior run counter to the available clinical data: m-CPP appears anxiogenic (Zohar et al. 1987), whereas ritanserin has been reported to have anxiolytic efficacy (Ceulemans et al. 1985).

Co-administration of ritanserin and agonists yielded different effects for DOI and m-CPP. The ritanserin blockade of DOI's effects on USV is consistent with the DOI effect being mediated by either the $5HT_2$ or $5HT_{1C}$ receptor. Co-administration of m-CPP and ritanserin resulted in a greater decrease in USV than observed following either drug alone. This latter result may reflect the emergence of other pharmacologic effects of m-CPP (i.e. $5HT_{1A}$ or adrenergic effects) in the presence of $5HT_2/5HT_{1C}$ receptor blockade (Hamik and Peroutka 1989). Indeed, rat pup USV have previously been shown to be exquisitely sensitive to drugs affecting adrenergic receptors (Hård et al. 1988; Winslow and Insel 1990a).

The effects of serotonergic drugs on ultrasonic vocal behavior do not appear to be secondary to effects on thermoregulation or respiration. Although previous reports in adult rats have detected hypothermia following 8-OH-DPAT (Goodwin et al. 1987; Wozniak et al. 1988) and hyperthermia following m-CPP (Wozniak et al. 1989), we were unable to detect significant changes in the core body temperature of infant rats with any of the substances tested. This difference between infant and adult rats in thermoregulatory response to serotonergic agents may be an another example of what Enters and Spear (1988) have called "ontogenetic transitions" in the behavioral response of infant rats to serotonergic drugs. In addition, the reduction of ultrasonic vocalization after $5HT_{1A}$ and $5HT_2$ agonists does not appear to be secondary to respiratory depression, as recent studies of serotonergic modulation of neonatal respiration suggest a generally stimulatory effect of 8-OH-DPAT and DOI (Morin et al. 1990).

In summary, consistent with previous reports, agonists at the $5HT_{1A}$ receptor mimicked the effects of several classes of anxiolytics in their reduction of ultrasonic vocal behavior of 9–11-day-old pups. In contrast to these $5HT_{1A}$-mediated effects, $5HT_{1B}$ receptor ligands generally increased ultrasonic vocalization. This anxiogenic-like effect of low doses of TFMPP and all doses of CGS12066B appears to be associated with the relatively high selectivity of these drugs for the $5HT_{1B}$ receptor, while the rate reducing effects of high doses of TFMPP and all doses of m-CPP and DOI appear to be more closely related to $5HT_2$ or $5HT_{1C}$ activity. Clarification of the $5HT_2$ and $5HT_{1C}$ mechanisms must await the development of highly selective $5HT_{1C}$ receptor antagonists. In an earlier study, we demonstrated that serotonin lesions significantly decreased rat pup ultrasonic vocalization (Winslow and Insel 1990b). These previous results, taken together with the current data using relatively selective receptor ligands, suggest a role for serotonin in the modulation of vocal behavior, based on a balance of inhibiting and facilitating receptor influences.

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