

Serotonergic modulation of the rat pup ultrasonic isolation call: studies with 5HT₁ and 5HT₂ 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]quinoline) 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 5HT₁ 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-methylphenyl)-2-aminopropane), drugs with putative actions at 5HT_{1C} and 5HT₂ receptor sites both decreased calling but differed according to their effects on motor activity. Ritanserin, a 5HT₂ 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 which receptor subtype is affected.

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 et 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 5HT₁ and 5HT₂ receptor subtypes.

Materials and methods

Subjects. Offspring of Sprague Dawley breeders (Taconic Farms, Germantown, New York) were housed with both parents in poly-

carbonate 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

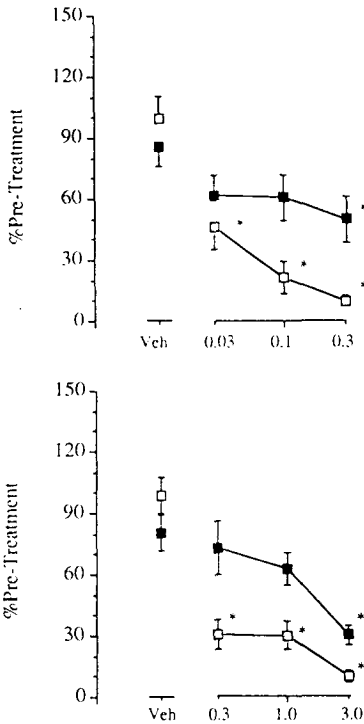


Fig. 1. The acute effects of buspirone (top) and 8-OH-DPAT (bottom) on the frequency of ultrasonic calling (open symbols) and grid cell crossing (closed symbols) during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test 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.

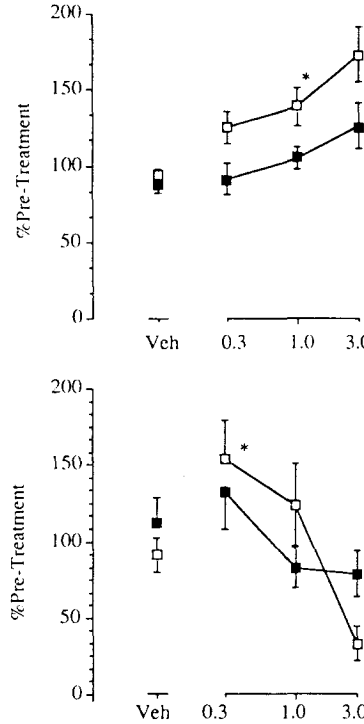


Fig. 2. The acute effects of CGS12066B (top), and TFMPP (bottom) on the frequency of ultrasonic calling (open symbols) and grid cell crossing (closed symbols) during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test 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.

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Table 1. 51

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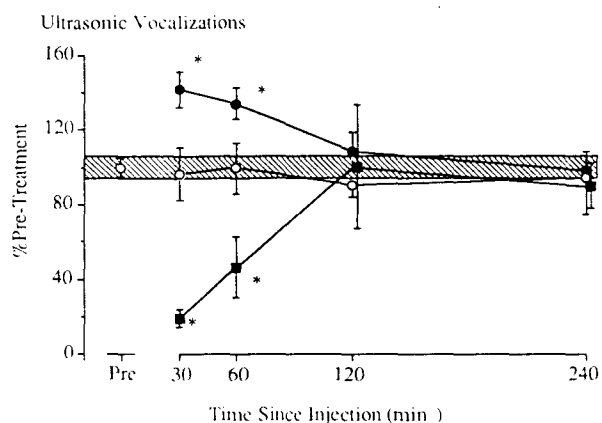


Fig. 3. The time course of 1.0 mg/kg buspirone (closed squares) and 1.0 mg/kg CGS12066B (closed circles) and saline (open circles) collected beginning 30 min after subcutaneous injection and measured in repeated 2-min tests at 60, 120, and 240 min after injection. Data are expressed as percent change 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_{1A}

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

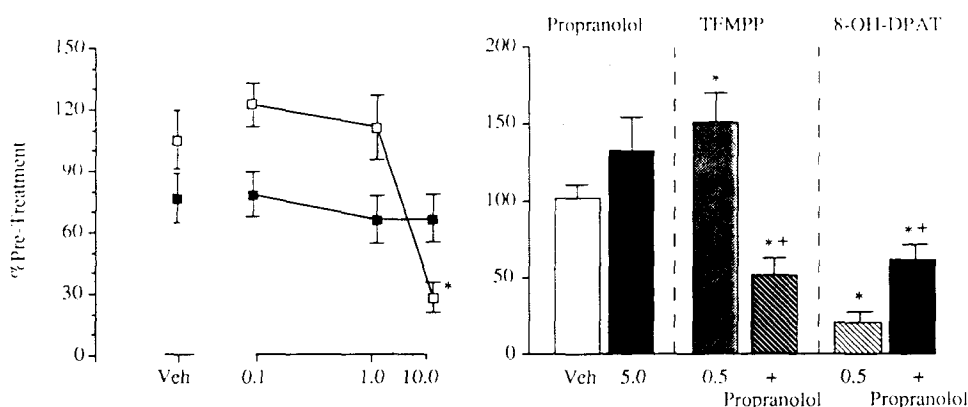


Fig. 4. The acute effects of *d,l*-propranolol (left panel) on the frequency of ultrasonic calling (open symbols) and grid cell crossing (closed symbols) during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test conducted 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 por-

trayed (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 ($P < 0.05$), pluses 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.

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Table 2. 51

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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}/5HT₂

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}/5HT₂ 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 ($t_D=2.11$, $P<0.05$)

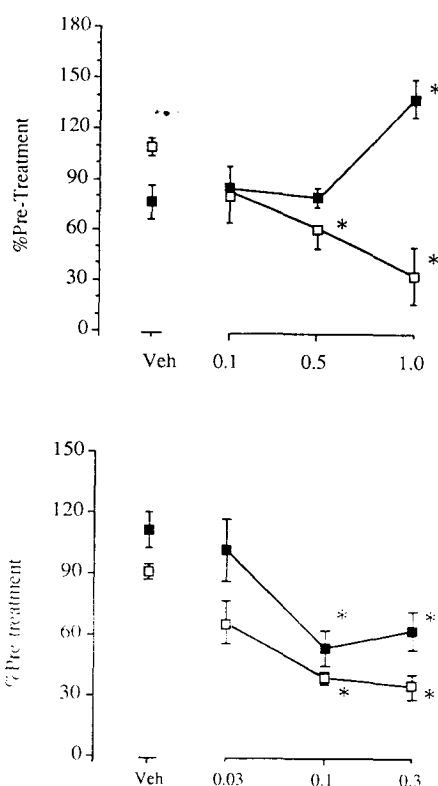


Fig. 5. The acute effects of m-CPP (top) and DOI (bottom) on the frequency of ultrasonic calling (open symbols) and grid cell crossing (closed symbols) during a 2-min separation beginning 30 min after subcutaneous injection. Data are expressed as percent change from a 2-min pre-injection test conducted immediately before drug injection. Vertical lines at each data point represent ± 1 SEM. Asterisks represent Dunnett's *t*-comparisons ($P<0.05$) in the presence of significant overall treatment effects detected by ANOVA.

Table 2. 5HT₂/5HT_{1C} Agonists and antagonists

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

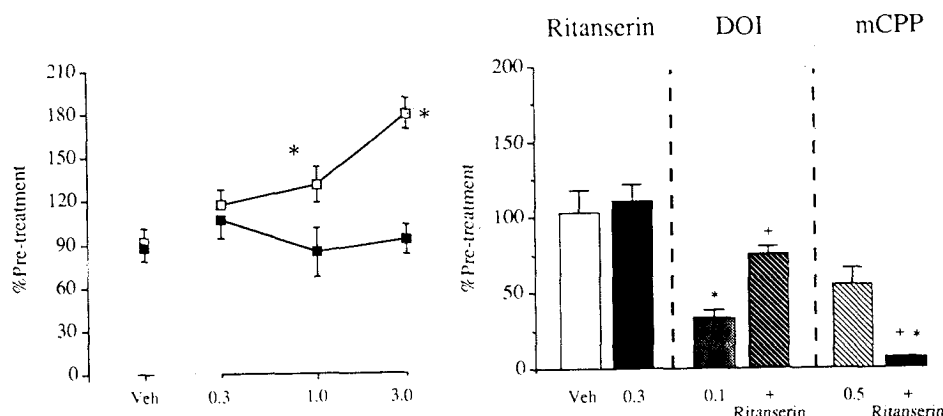


Fig. 6. The acute effects of ritanserin (*left panel*) on the frequency of ultrasonic calling (*open symbols*) and grid cell crossing (*closed symbols*) during a 2-min separation beginning 30 min after subcutaneous injection. Also presented (*right panel*) are the effects of co-administration of ritanserin (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 ($t_b = 3.51$, $P < 0.05$).

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 Eriksson 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 (Broekamp 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

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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 5HT₂/5HT_{1C} mediated rate decreasing effects which emerged at higher doses.

The 5HT₂/5HT_{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 5HT₂/5HT_{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 5HT₂ 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 5HT₂/5HT_{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 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 5HT₂ 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 5HT₂ or 5HT_{1C} activity. Clarification of the 5HT₂ 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.

References

- Benjamin D, Lal H, Meyerson LR (1990) The effects of 5HT_{1B} characterizing agents in the mouse elevated plus-maze. *Life Sci* 47: 195–203
- Berendsen HH, Broekkamp CL (1987) Drug-induced penile erections in rats: indications of serotonin receptor mediation. *Eur J Pharmacol* 135: 279–287
- Broekamp C, Jenk F (1989) The relationship between various animal models of anxiety, fear-related psychiatric syndromes and response to serotonergic drugs. In: Bevan P, Cools A, Archer T (eds) *Behavioural pharmacology of 5H-T*. Lawrence Erlbaum, Hillsdale, NJ, pp 321–337
- Burkholder JH, Hill JL, Vaughn WJ, Casio HE (1982) A broadband digitizing rat detector: simultaneous recording from all sound frequencies in the range of rat ultrasonic vocalizations. *Behav Res Meth Instr* 14: 511–518
- Ceulemans DLS, Hoppenbrouwers M, Gelders YG, Reyntjens AJM (1985) The influence of ritanserin, a serotonin antagonist, in anxiety disorders. A double-blind placebo-controlled study versus lorazepam. *Pharmacopsychiatry* 18: 303–305
- Curzon G, Kennett GA (1990) mCPP: a tool for studying behavioural responses associated with 5-HT_{1C} receptors. *TIPS* 11: 181–182
- Engel G, Gother M, Hoyer D, Schlicker E, Hillenbrand K (1986) Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Arch Pharmacol* 332: 1–7
- Enters EK, Spears LP (1988) Ontogenetic transitions in the psychopharmacological response to serotonergic manipulations. *Psychopharmacology* 96: 161–168
- Eriksson E, Humble M (1990) Serotonin in psychiatric pathophysiology: a review of data from experimental and clinical research. In: Pohl R, Gershon S (eds) *The biological basis of psychiatric treatment. Progress in basic and clinical pharmacology*. Karger, Basel, pp 66–119
- Gardner CR (1985) Inhibition of ultrasonic distress vocalizations in rat pups by chlordiazepoxide and diazepam. *Drug Dev Res* 5: 185–193
- Gardner CR, Buddhram P (1987) Effects of agents which interact with central benzodiazepine binding sites on stress. *Eur J Pharmacol* 134: 275–283
- Goodwin GM, De Souza RJ, Green AR, Heal DJ (1987) The pharmacology of the behavioral and hypothermic responses of rats to 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). *Psychopharmacology* 91: 506–511
- Hamik A, Peroutka SJ (1989) 1-(m-Chlorophenyl)piperazine (mCPP) interactions with neurotransmitter receptors in the human brain. *Biol Psychiatry* 25: 569–575
- Hamon M, Lanfumey L, El Mestikawy S, Boni C, Miquel M-C, Bolanos F, Schecter L, Gozlan H (1990) The main features of central 5-HT receptors. *Neuropsychopharmacology* 3: 349–360
- Hård E, Engel J (1988) Effects of 8-OH-DPAT on ultrasonic vocal-

- ization and audiogenic immobility reaction in pre-weaning rats. *Neuropharmacology* 27:981-986
- Hård E, Engel J, Lindh A-S (1988) Effect of clonidine on ultrasonic vocalization in preweaning rats. *J Neural Transm* 73:217-237
- Hartig PR, Hoffman BJ, Kaufman MJ, Hirata F (1990) The 5-HT_{1C} receptor. In: Whitaker-Azmitia PM, Peroutka SJ (eds) *The neuropharmacology of serotonin*. The New York Academy of Sciences, New York, pp 149-167
- Hofer MA, Shair HN (1987) Isolation distress in two-week-old rats: influence of home cage, social companions, and prior experience with littermates. *Dev Psychobiol* 20:465-476
- Hofer MA, Shair HN, Murowchick E (1989) Isolation distress and maternal comfort responses of two-week-old rat pups reared in social isolation. *Dev Psychobiol* 22:553-556
- Hutson PH, Kennett GA, Donohoe TP, Dourish CT, Curzon G (1989) Opposite effects of 5HT_{1A} and 5HT_{1B/1C} agonists on food intake. In: Bevan I, Cools AR, Archer T (eds) *Behavioral pharmacology of 5-HT*. Lawrence Erlbaum, Hillsdale, NJ, pp 283-287
- Insel T, Winslow J (1990) Neurobiology of obsessive-compulsive disorder: a possible role for serotonin. *J Clin Psychiatry* 51:1-9
- Insel TR, Winslow JT (1991) Rat pup ultrasonic vocalizations: an ethologically relevant behavior responsive to anxiolytics. In: Olivier B, Mos J (eds) *New animal models of psychiatric illness* (in press)
- Insel TR, Hill JL, Mayor RB (1986) Rat pup ultrasonic isolation calls: possible mediation by the benzodiazepine receptor complex. *Pharmacol Biochem Behav* 24:1263-1267
- Insel TR, Miller LP, Gelhard RE, Hill JL (1988) Rat pup ultrasonic isolation calls and the benzodiazepine receptor. In: Newman JD (ed) *The physiological control of mammalian vocalization*. Plenum Press, New York, pp 331-342
- Kehoe P (1988) Ontogeny of adrenergic and opioid effects on separation vocalizations in rats. In: Newman J (ed) *The physiological control of mammalian vocalization*. Plenum Press, New York, pp 301-320
- Kehoe P, Blass EM (1986) Opioid-mediation of separation distress in 10-day-old rats: reversal of stress with maternal stimuli. *Dev Psychobiol* 19:385-398
- Kennett G, Whitton P, Shah K, Curzon G (1989) Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. *Eur J Pharmacol* 164:445-454
- Middlemiss DN, Hutson PH (1990) The 5-HT_{1B} receptors. In: Whitaker-Azmitia PM, Peroutka SJ (eds) *The neuropharmacology of serotonin*. The New York Academy of Sciences, New York, pp 132-149
- Morin D, Hennequin S, Monteau R, Hilaire G (1990) Serotonergic influences on central respiratory activity: an in vitro study in the newborn rat. *Brain Res* 535:281-287
- Mos J, Olivier B (1989) Ultrasonic vocalizations by rat pups as an animal model for anxiolytic activity: effects of serotonergic drugs. In: Archer T, Bevan PAC (eds) *Behavioural pharmacology of 5-HT*. Lawrence Erlbaum, Hillsdale, NJ, pp 361-366
- Neale RF, Fallon SL, Boyar WC, Wasley JWF, Martin LL, Stone GA, Glaeser BS, Sinton CM, Williams M (1987) Biochemical and pharmacological characterization of CGS12066B, a selective serotonin-1B agonist. *Eur J Pharmacol* 136:1-9
- Noirot E (1966) Ultra-sounds in young rodents. I. Changes with age in albino mice. *Anim Behav* 14:459-462
- Noirot E (1972) Ultrasounds and maternal behavior in small rodents. *Dev Psychobiology* 5:5-21
- Renyi L, Lewander T (1989) 5-HT_{1A} and 5-HT_{1B} agonists produce opposite effects on the ejaculatory response induced by amphetamine in rats. In: Bevan I, Cools AR, Archer T (eds) *Behavioral pharmacology of 5-HT*. Lawrence Erlbaum, Hillsdale, NJ, pp 79-83
- Shannon M, Battaglia G, Glennon RA, Titeler M (1984) 5HT₁ and 5HT₂ binding properties of derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). *Eur J Pharmacol* 102:23-29
- Smootherman WP, Bell RW, Starzec I, Elias J, Zachman TA (1974) Maternal responses to infant vocalizations and olfactory cues in rats and mice. *Behav Biol* 12:55-66
- Soubrie P (1986) Reconciling the role of central serotonin neurons in human and animal behavior. *Behav Brain Sci* 9:319-364
- Taylor DP (1987) Buspirone, a new approach to the treatment of anxiety. *FASEB* 2:2445-2552
- Titeler M, Lyon RA, Davis KH, Glennon RA (1987) Selectivity of serotonergic drugs for multiple brain serotonin receptor: the role of ³H DOB, a 5-HT₁ agonist radioligand. *Biochem Pharmacol* 36:3265-3271
- Titeler M, Lyon RA, Glennon RA (1988) Radioligand binding evidence implicates the brain 5-HT₂ receptor as the site-of-action for LSD and phenylisopropylamine hallucinogens. *Psychopharmacology* 94:213-216
- Winer BJ (1971) *Statistical principles in experimental design*. McGraw-Hill, New York
- Winslow JT, Insel TR (1990a) Serotonergic and catecholaminergic reuptake inhibitors have opposite effects on the ultrasonic isolation call of rat pups. *Neuropsychopharmacology* 3:51-59
- Winslow JT, Insel TR (1990b) Serotonergic modulation of rat pup ultrasonic vocal development: studies with MDMA. *J Pharmacol Exp Ther* 254:212-220
- Winslow JT, Insel TR (1991) Endogenous opioids: do they mediate the rat pup's response to social isolation? *Behav Neurosci* 105:31-41
- Winslow JT, Insel TR, Trullas R, Skolnick P (1990) Rat pup isolation calls are reduced by functional antagonists of the NMDA receptor complex. *Eur J Pharmacol* 190:11-21
- Wozniak KM, Aulakh CS, Hill JL, Murphy DL (1988) The effect of 8-OH-DPAT on temperature in the rat and its modification by chronic antidepressant treatments. *Pharmacol Biochem Behav* 30:1:451-456
- Wozniak KM, Aulakh CS, Hill JL, Murphy DL (1989) Hyperthermia induced by m-CPP in the rat and its modification by antidepressant treatments. *Psychopharmacology* 97:269-274
- Zohar J, Mueller EA, Insel TR, Zohar-Kadouch RC, Murphy DL (1987) Serotonergic responsivity in obsessive-compulsive disorder: comparison of patients and healthy controls. *Arch Gen Psychiatry* 44:946-951

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