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PRX-00023, a selective serotonin 1A receptor agonist, reduces ultrasonic vocalizations in infant rats bred for high infantile anxiety

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ABSTRACT

To address the development of early anxiety disorders across the lifespan, the High USV line of rats was bred based on rates of infant ultrasonic vocalization in the 40–50 kHz range of predominant frequencies (USV) to maternal separation at postnatal day (P) 10. In this study, rates of USV in High line infants (pups: Postnatal Day 11 ± 1) were compared to those of randomly-bred controls in response to EPIX compound PRX-00023, a unique serotonin (5-HT) agonist, acting exclusively at the 5-HT1A receptor, or buspirone, a nonspecific 5HT1A agonist. After testing, pups were examined for sedation and other drug-related effects. The results indicated that all doses of buspirone reduced USV rates in isolation, consistent with other reports. PRX-00023 significantly reduced USV rates at the lowest doses (0.01–0.05 mg/kg). None of the PRX-00023 doses produced sedation, whereas all but the lowest dose of buspirone (0.1 mg/kg) produced sedation effects. The results suggest that this compound alleviates infantile anxiety-like behavior with great specificity in rats bred for high anxiety/depressive phenotypes by selectively targeting 5-HT1A receptors, possibly by both pre- and post-synaptic mechanisms.

1. Introduction

Anxiety is one of the most common and disabling psychiatric disorders among adults and children (Baldwin and Polkinghorn, 2005).The developmental origins of anxiety may have links to genetic factors that have been shown to have a role in anxiety disorders (Weissman et al., 1997). Family genetic-association studies show that anxiety disorders are elevated in children of parents with anxiety and depressive disorders (Weissman et al., 2006). Moreover, higher rates of anxiety disorders in childhood predict both anxiety and depressive disorders in adulthood, and it is more likely that these adults will pass on the disorder to their children, thus completing the cycle (Schwartz et al., 1999; Weissman et al., 2006).

Decades of pharmacological studies have demonstrated that ultrasonic cries in the 40–50 kHz range of predominant frequencies in separated, isolated rat pups reflect a primitive form of infantile anxiety; these cries are potently reduced by drugs targeting the serotonin (5-hydroxytryptamine, or 5-HT) system (see Brunelli and Hofer, 2001 for review). Recent studies have also shown that USVs are not by-products of either attempts to increase or reduce thermal effects of cold or to increase blood pressure and blood flow to the heart (Blumberg et al., 1992; Blumberg et al., 2001; Hofer and Shair, 1993; Shair and Jasper, 2003).

As a model for inherited anxiety disorders having their first expression in childhood, this laboratory has bred adult rats for high or low rates of cries in the ultrasonic (40–50 kHz) range in infancy in response to separation from the mother (dam) in a novel environment (Brunelli, 2005). As shown in Fig. 1 over 20 generations, High line infant USV rates rose 4-fold over randomly-bred (Random line) rats and these rates have been maintained consistent with the hypothesis that the USV phenotype is highly heritable. The developmental continuity of affective dysregulation in High line adults has been demonstrated as anxiety and depression-like behaviors in standard laboratory tests (Brunelli and Hofer, 2007; Zimmerberg et al., 2005). In this respect, High line infant and adult phenotypes resemble those found in human family-genetic

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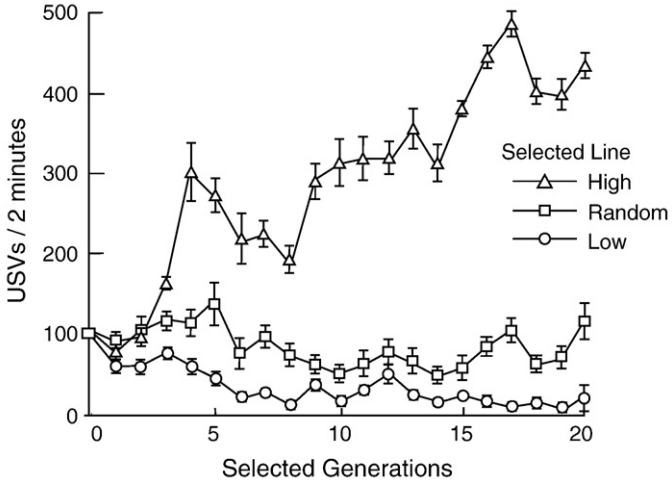

Fig. 1. Results of selective breeding across 20 generations in High, Low and Random (control) lines. The high or low phenotype selected was based on the total number of USVs emitted during 2-minutes isolation at room temperature. The High line is indicated by filled triangles, the Low line by open circles, and the Random line by filled squares. Values are for litter means: the number of litters tested per generation in each line ranged from: 14–39 (median = 28) for Highs; 17–32 (median = 22) Randoms 14–44 (median = 28.5) Lows. With permission from Behavior Genetics, 2005; 35(1): 53–65.

studies, in that child anxiety phenotypes tend to resolve into depressive disorders in adulthood (Leonardo and Hen, 2008; Weissman et al., 2006).

The serotonin system has long been known to be involved in affective regulation in humans and animals. Selective serotonin reuptake inhibitors (SSRIs) are first-line treatment medications for depression and anxiety disorders in adults and children. Although generally well tolerated, a small percentage of children and adolescents suffer from adverse neuropsychiatric effects such as behavioral activation (hyperactivity, irritability, impulsivity, talkativeness), and SSRIs may even increase suicidal ideation and behavior in some adolescents (Hammad, 2004; Hammad et al., 2006; Kaizar et al., 2006; Murphy et al., 2008; Olfson et al., 2003; Zuckerman et al., 2007). The azapirones such as buspirone, another class of anxiolytics targeting the 5-HT1A serotonin receptors, have also been used as anxiolytics in adults and in children with generalized anxiety disorder (Allgulander et al., 2003; Hidalgo et al., 2007). Although buspirone is generally safe and well tolerated in adults, a large, multi-site study of its pharmacokinetics and tolerability found that a high percentage of children and adolescents reported one or more adverse events such as lightheadedness (68%), headache (48%), and dyspepsia (20%) at low doses, and other adverse effects leading to study termination at higher doses (Salazar et al., 2001). Thus, children and adolescents appear to be highly sensitive to the adverse effects of both SSRIs and azapirones.

PRX-00023 is a highly selective 5-HT1A agonist (IC50 ~ 20 nM) that is not a member of the azapirone class and has minimal alpha-adrenergic cross-reactivity. PRX-00023 was developed *in silico* by 3-dimensional computer modeling (Becker et al., 2006). In clinical trials PRX-00023 has proven to be safe and well-tolerated in adults in doses ranging from 10 mg/kg to 150 mg/k (Iyer et al., 2007; Rickels et al., 2008). Preclinical tests of adult rodents show that PRX-00023 potently reduces depressive- and anxiety-like behavior with a high degree of tolerability (Becker et al., 2006; De Paulis, 2007). However, its effects in infant anxiety models like USV are as yet completely unknown.

In this study it was hypothesized that PRX-00023 would act potently to reduce High line pup USV rates with few, if any other effects on behavior or physiology. It was also hypothesized that the High line would be more sensitive to the anxiety-reduction effects of 5-HT1A agonists compared to the Random line. If PRX-00023 was found to reduce high rates of USV in High line rat pups without harmful side effects it could potentially be a new treatment for anxiety disorders in children.

2. Methods

2.1. Subjects

Twenty-four litters of 5–8 pups in each strain of rats (High and Random) were tested for USV rates in isolation. Birth was designated as Postnatal Day (P) 0. Within 48 h after birth, all litters were culled to 8 pups (range 4–9) with equal numbers of females and males as possible. Overall, 510 pups were used. Animals were housed in polyethylene cages (40 × 20 × 24 cm) with shavings and provided with food and water ad lib in the temperature- and humidity-controlled animal quarters, in a 12:12 h light /dark cycle (lights on from 0600–1800 h).

2.2. Drugs and doses

PRX-00023 and buspirone were supplied by the EPIX Corporation (Lexington, Massachusetts). Drugs were dissolved in saline vehicle prior to injections. Each pup was injected in the intraperitoneal space (i.p.) with one of several doses of PRX-00023 (0.01, 0.03, 0.05, 0.1, 0.3, 1.0, and 3.0 mg/kg in saline, for a total volume of 0.1 mg/kg). Within each litter two littermates, though occasionally one, received the same dose of a compound. Because of the distribution of pups in litters used, no Random line pups were tested with PRX-00023 at 3.0 mg/kg for comparison to vehicle.

Buspirone was used as the positive control for PRX-00023. Buspirone is a 5-HT1A agonist, characterized by rapid absorption, low oral bioavailability due to extensive first-pass metabolism, and a short half-life of 2 to 4 h (Edwards et al., 2006). Buspirone also has activities at other receptors (e.g., the α-adrenergic receptor and dopamine D2 receptor) that cause effects such as nausea, headache, nervousness, restlessness and dizziness (Becker et al., 2006). Buspirone has also been shown to have potent sedative properties in infant rats and mice, as shown by the righting reflex and latencies to turn upright on an inclined plane (Kehne et al., 1991).

The doses of buspirone chosen (0.1, 0.3, and 1.0, 3.0 mg/kg, in a volume of 0.1 ml saline/gram pup weight) were based on a range of its effects on USV and other measures tested in separated rat pups (Olivier et al., 1998; Mos and Olivier, 1989). In adult rats both buspirone and PRX-00023 achieve their anxiolytic effects at doses ranging from 1 to 20 mg/kg (De Paulis, 2007). Saline was used as a vehicle control.

2.3. USV test procedure

Testing for isolation-induced USV was done on P11 (± 1 day), between 1000 and 1600 h. The dam was removed from the home cage 20 min before testing, and the covered home cage containing the litter of pups was carried into the test room. A thermostatically controlled heat source was placed under the home cage floor to maintain pup temperature at normal nest levels (35–37 °C). Because USV rates are influenced by ambient and body temperature, and because buspirone reduces pup temperature in a dose–response fashion (Olivier et al., 1998), pups' temperatures were taken in the home cage before injection; 30 min after injection, immediately before being placed into the test cage; and immediately after testing, to observe the magnitude of this effect in the two drugs. The protocol used is as follows. Just before injection, each pup was picked up and axillary temperature (pre-injection temperature) was taken by gentle insertion of the side of a microprobe (Physitemp I-18 attached to a Physitemp BAT-10 digital thermometer) in the skin pocket between the upper foreleg and chest wall of the animal. The pup was then immediately weighed and injected with one drug dose by weight (0.1 mg/kg of pup weight) and then marked with a non-toxic permanent marker for identification. The animal was then replaced in the litter pile. After a 30-minute post-injection interval, the pup was picked up off the litter pile, the

pre-test axillary temperature was taken as before and then the pup was placed immediately into a test chamber (18 cm × 20 cm × 21 cm) with the floor marked in a grid of six equal squares. After the test, the pup's post-test axillary temperature was measured as before; the ambient temperature of the test cage was also measured. Pups were tested in the order in which they were injected. Pups were tested in the chamber for a total of 2 m. Pilot data have shown a high, ($r = 0.86$, p, 0.01), correlation between USV totals at 2 and at 6 min, indicating stability in this response. The test room temperature varied (21–23 °C) within the limits allowed by the laboratory building's heating system (Brunelli et al., 1997; Wiedenmayer and Barr, 2001).

2.4. Ultrasound detection and behavioral measurement

To detect USVs, a microphone attached to a bat detector set in the broadband mode was suspended 20 cm above the cage floor (Pettersson Elektronik AB, Sweden). This circuitry responds to the dominant frequency in the 10–100-kHz range, transducing it to an audible signal, and does not depend on prior tuning to a specific frequency. Earphones were used to avoid feedback effects on pups. Each instance of these audible calls was counted by pressing a button that activated a soundless electric counter. A second observer noted the cumulative number of those USV counts every 15 s. This method has been highly reliable for over more than 25 years of testing with an inter-observer correlation in the range of $r = 0.95$–0.97 (Brunelli et al., 1996; Hofer and Shair, 1978; Brunelli, unpublished data). USV rates counted by hand are also highly correlated with electronically recorded measurements of USV rate ($r \geq 0.98$, unpublished data preparatory to studies by Myers et al., 2004). Similarly, each instance of a behavior measured was identified by the first observer and noted by the second observer within the same 15-s time frame. These measures consisted of the number of rears, face washes, defecation/urination, and number of squares crossed in the test cage (as a measure of activity) by the pup. The frequency of freezing during isolation is extremely low; in our experience too low to detect differences between groups outside of the context of exposure to strange adult males (see Brunelli et al., 1998). Freezing noted anecdotally in isolation was virtually absent, and was therefore not reported.

Since at P11 (± 1 day) pups do not have full use of their hind legs, a second measure of activity called "turn in square" was counted, in which the pup makes a 360-degree pivot of the front part of the body around its haunches (Brunelli et al., 1997). As measures for side effects of drugs, the presence or absence of head-weaving was noted (Fish et al., 2000; Darmani and Ahmad, 1999; Jackson and Kitchen, 1989; Shayit et al., 2003).

2.5. Negative geotaxis

Two negative geotaxis behaviors were used as measures of sedation (Fish et al., 2000). First, immediately after post-isolation axillary temperature measurement, each pup was placed on its back and the latency (in tenths of seconds) to flip onto its ventrum was recorded. Then each pup was placed head-down at a 30° angle on an inclined plane covered with fine-gauge screening. Second, the latency to turn 180° around from a head-down to a head-up position was measured, with a cut-off time of 60 s. After these two tests, pups were placed back onto the litter pile. The presence or absence (as 1/0) of ultrasonic vocalizations was noted in each pup during these negative geotaxis tests. All procedures were done with the approval of New York State Psychiatric Institute Institutional Animal Care and Use Committee.

2.6. Data analysis

Data recorded during testing were entered into a database program (SYSTAT: Wilkinson, 1997). After initial tests for the effects of sex on weight and USV rates in individual pups (see Results), means were taken of each pair of same-sex pups injected with the same dose of drug within each litter. Multivariate ANOVA analyzed main effects of selected line and drug conditions, with sex, weight and age covaried, followed by post-hoc Dunnett comparisons between vehicle and drug conditions (Systat Version 11.0) For line × drug interactions, within each line one-way ANOVAs, followed by post-hoc Dunnett comparisons tested the effects of each drug dose against vehicle. Yates-corrected Chi Square was used to test for differences in the frequencies of ordinal variables such as the presence/absence of vocalizations on the inclined plane, of head-weaving, and of in-coordination during testing.

3. Results

3.1. Pup weights

Preliminary to analysis of USV, a 3-way ANOVA analyzed the independent effects of Age, Line and Sex on individual pup weights, using litter as a covariate. The 3-way model indicated significant main effects of Line, $F(1,502) = 60.66$, $p < 0.01$; Sex, $F(1,502) = 5.16$, $p = 0.02$; Age, $F(2, 502) = 16.32$, $p < 0.01$; and a Line × Age interaction, $F(2,502) = 3.00$, $p = 0.05$, with litter as a significant covariate, $F(1,502) = 32.48$, $p < 0.001$. Random line pups weighed more than High line pups; male pups weighed more than female pups; pups at 10 days of age weighed less than pups at 11 and 12 days of age (Dunnett $ps < 0.001$). With respect to pup age, a similar analysis found no Line, Sex or Line × Sex interaction effects; however, litter was a significant covariate, indicating significant differences in litter weights independent of other effects. Therefore, mean litter values for each sex and drug condition were used as the unit for all subsequent analyses (Zorrilla, 1997). Because weight can have direct effects on body temperature and therefore indirectly on other behaviors in isolation (Brunelli and Hofer, 1996), the effects of weight, age and sex were tested as covariates in all subsequent ANOVAs.

3.2. Pup temperatures

A repeated measures ANOVA for pre-injection, pre-test, and post-test axillary temperatures indicated a significant effect of Drug Condition, $F(8,387) = 7.74$, $p < 0.001$ (Table 1). There were no significant main effects of Line or interactions with Line. A significant within-subjects effect, $F(2,774) = 29.12$, $p < 0.001$, indicated that pre-injection axillary temperatures were significantly higher than pre-test and post-test axillary temperatures. A significant within-subjects interaction with Drug Condition, $F(16, 770) = 8.98$, $p < 0.001$, indicated that buspirone reduced pre- and post-axillary temperatures as dosage increased (Fig. 2a). A significant within-subjects interaction with Age, $F(2,386) = 6.96$, $p < 0.01$, indicated that for 10-day-old pups, pre- and post-test axillary temperatures were significantly lower than 11- and 12-day-old pups, $ps < 0.01$, and similarly 11-day-old pup pre-/post-axillary pup axillary temperatures were lower than 12-day-old pups, $ps < 0.01$ (Fig. 2b). On its own, weight was a significant contributor to axillary temperature, $F(1,387) = 85.05$, $p = 0.001$, so that smaller pups had lower pre-injection axillary temperatures than heavier pups regardless of Line or Drug Condition (Table 1). A significant within-subject interaction with weight, $F(2,386) = 14.04$, $p < 0.01$, indicated that pre-injection axillary temperature was less affected by weight than pre-test and post-test axillary temperatures.

3.3. USV rates

(Table 2). Because PRX-00023 at doses of 0.01, 0.03 and 0.05 mg/kg did not differ from one another in their effects on USV rates in either line, these three dosages were combined into one to increase power to detect differences in USV rates. In addition, because no Random and only 2 High line litters were tested at PRX-00023, 3.0 mg/kg, this group was dropped from analysis.

Table 1
Weight and temperature measures: mean (sem).

Line	Drug condition mg/kg	Sample *n*	Age	Weight	Axillary temperatures			
					Pre-injection	Pre-test	Post-test	Ambient
High	Vehicle	45	11.02 (0.12)	22.43 (0.56)	35.47 (0.11)	35.81 (0.82)	34.52 (0.14)	22.58 (0.14)
	Buspirone 0.1	40	10.88 (0.11)	22.42 (0.61)	35.33 (0.17)	35.60 (0.14)	34.50 (0.13)	22.43 (0.17)
	Buspirone 0.3	14	10.64 (0.23)	20.36 (0.90)	36.10 (0.18)	35.02 (0.30)	34.00 (0.28)	23.59 (0.89)
	Buspirone 1.0	16	10.94 (0.23)	21.35 (0.85)	35.64 (0.28)	34.58 (1.51)	33.34 (0.40)	22.22 (0.28)
	Buspirone 3.0	4	10.00 (0)	16.17 (0.32)	35.42 (0.20)	31.67 (0.20)	31.12 (0.22)	23.82 (0.14)
	PRX	39	11.21 (0.11)	24.19 (0.57)	35.35 (0.17)	35.82 (0.20)	34.58 (0.19)	22.34 (0.13)
	PRX 0.1	32	11.06 (0.12)	22.70 (0.76)	35.39 (0.24)	35.76 (0.17)	34.63 (0.19)	22.28 (0.14)
	PRX 0.3	31	11.13 (0.13)	23.42 (0.68)	35.46 (0.22)	35.59 (0.19)	34.57 (0.21)	
	PRX 1.0	13	10.46 (0.18)	21.10 (0.80)	35.14 (0.34)	35.55 (0.15)	33.89 (0.36)	22.00 (0.44)
Random	Vehicle	32	10.88 (0.15)	24.78 (0.93)	35.70 (0.12)	35.81 (0.21)	34.72 (0.20)	22.63 (0.18)
	Buspirone 0.1	29	11.00 (0.15)	24.34 (0.79)	35.62 (0.17)	35.79 (0.18)	34.73 (0.16)	22.55 (0.18)
	Buspirone 0.3	4	10.00 (0)	23.20 (0.99)	35.58 (0.15)	34.13 (0.26)	33.49 (0.21)	23.48 (0.41)
	Buspirone 1.0	5	10.00 (0)	23.87 (0.62)	35.60 (0.15)	33.26 (0.84)	32.49 (0)	23.13 (0.43)
	Buspirone 3.0	4	10.00 (0)	25.40 (1.00)	35.59 (0.16)	32.75 (0.15)	31.75 (0.15)	23.13 (0.43)
	PRX	32	11.34 (0.12)	26.25 (0.79)	35.66 (0.11)	36.18 (0.10)	35.22 (0.10)	22.41 (0.20)
	PRX 0.1	29	11.00 (0.15)	24.34 (0.79)	35.62 (0.17)	35.79 (0.18)	34.73 (0.16)	22.55 (0.18)
	PRX 0.3	31	11.19 (0.03)	24.92 (0.86)	35.62 (0.15)	36.09 (0.16)	34.78 (0.18)	22.40 (0.16)
	PRX 1.0	9	10.67 (0.24)	24.65 (1.93)	35.28 (0.22)	35.60 (0.23)	34.43 (0.34)	22.22 (0.20)

A multivariate ANOVA Line effect, $F(1,387)=61.60$, $p<0.001$, indicated that High line pup rates of USV were significantly higher than Random line overall. A significant effect of Drug Condition, $F(8,387)=6.00$, $p<0.001$, and a significant Line×Drug interaction, $F(8, 387)=2.31$, $p=0.02$, showed differences in the degree to which each drug dose affected USV rates in the High and Random lines. There were no significant effects of Age, Sex or Weight as covariates.

Next, ANOVAs tested effects of drug dosages within each line separately. As shown in Fig. 3a, High line USV rates were affected by Drug Condition, $F(8,222)=8.05$, $p<0.001$. There were no effects of Age or Sex, but weight covaried significantly, $F(1,222)=7.19$, $p=0.01$. Post-hoc Dunnett comparisons between vehicle and drugs showed that all doses of buspirone ($ps<0.01$) and the lowest doses of PRX-00023 significantly reduced High line USV rates below those of vehicle controls ($p<0.05$). Higher doses of PRX-00023 had no effect on USV rates.

In Random line pups, the main effect of Drug was not significant $F(8,162)=1.63$, $p=0.12$ (Fig. 3b). Power analysis calculations indicated that a minimum of 18 to 30 subjects (i.e., litter means for a given drug dose) would have been necessary to obtain multivariate effect sizes between 0.54 and 0.80. Several drug dose groups (i.e., buspirone 0.3–3.0 mg/kg and PRX-00023, 1.0 mg/kg) in the Random line sample did not meet the minimum criterion. Therefore, *t*-tests explored the effect of each drug dose on USV compared to vehicle USV rates individually, using relaxed alpha criteria. These revealed a trend for buspirone at 0.1 mg/kg, $t(37.5)=-1.705$, $p<0.10$, and a significant effect of buspirone at 0.3 mg/kg, $t(32.3)=-2.911$, $p<0.01$, to reduce USV rates below vehicle level in Random line pups. This was not true of any PRX-00023 dose even though cell numbers were sufficiently large to detect a univariate effect. Aside from drug dosage, age was a significant covariate effect, $F(1,162)=4.05$, $p=0.05$, indicating that USV rates were lower in 12-day-old pups than 11-day-old pups, $p=0.01$.

3.4. Behavioral variables

High and Random line pups did not differ in number of defecation/urinations. A significant Drug effect, $F(8,387)=3.47$, $p<0.01$, showed that 3.0 mg/kg buspirone increased defecations in both lines. A trend for Line, $F(1,387)=3.23$, $p=0.07$, indicated more face-washing in Random line pups than High pups. Heavier pups rose on hind legs more, $F(1,387)=20.85$, $p<0.01$, and older pups crossed more squares in the cage, $F(1,387)=4.46$, $p<0.05$. Drug condition significantly affected turns-in-square, $F(1,387)=2.42$. $p=0.01$, which was decreased by buspirone at 0.1, 0.3 and 1.0 mg/kg compared to vehicle, s = 0.04, 0.11 and 0.05, respectively (Table 2).

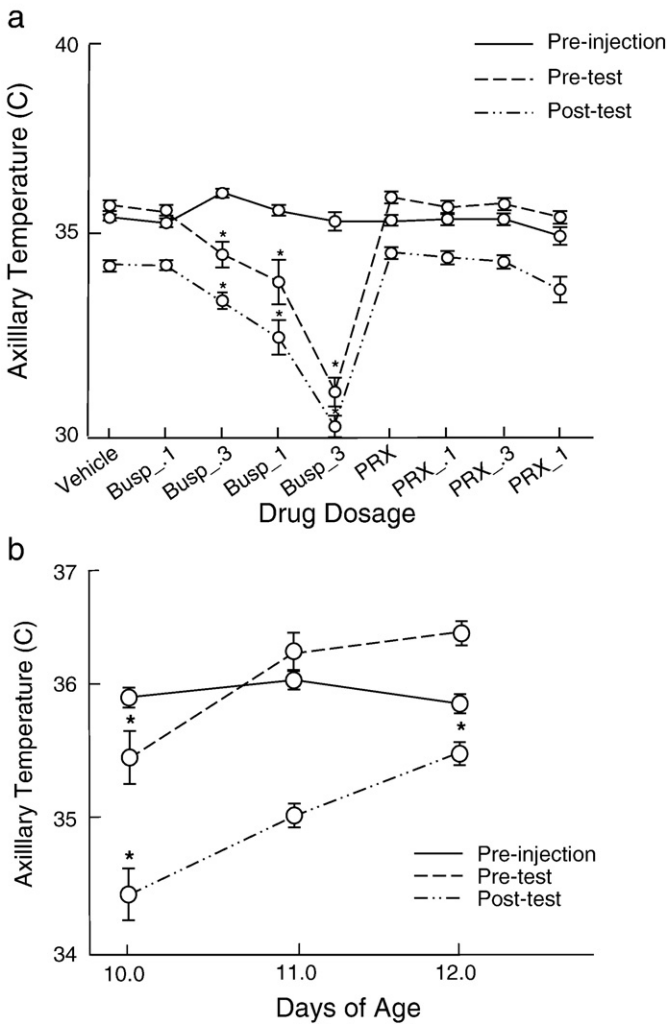

Fig. 2. Axillary temperature variation (PRX = PRX-00023; Busp = buspirone). * $p<0.05$, ~$p<0.10$. a. By drug dosage. b. By age.

Table 2
USV and behavior measures: mean (sem).

Line	Drug condition mg/kg	Sample *n*	USV rate	Defecate/urinate	Face wash	Rise	Square cross	Turn-in-square
High	Vehicle	45	288.70 (21.03)	0.23 (0.08)	0.58 (0.15)	0.53 (0.24)	1.86 (0.37)	0.73 (0.16)
	Buspirone 0.1	40	164.56 (24.89)	0.41 (0.12)	0.79 (0.14)	0.69 (0.21)	2.44 (0.51)	0.45 (0.14)
	Buspirone 0.3	14	89.34 (36.60)	0.75 (0.32)	0.36 (0.13)	0.46 (0.21)	2.50 (0.66)	0.36 (0.20)
	Buspirone 1.0	16	67.92 (27.43)	0.59 (0.15)	0.62 (0.20)	0.28 (0.17)	1.75 (0.41)	0.25 (0.14)
	Buspirone 3.0	4	4.12 (1.48)	1.00 (0.58)	0 (0)	0 (0)	2.25 (0.85)	0 (0)
	PRX	39	212.90 (19.80)	0.26 (0.08)	0.60 (0.12)	0.80 (0.23)	1.64 (0.24)	0.27 (0.07)
	PRX 0.1	32	241.66 (25.20)	0.22 (0.09)	0.94 (0.17)	0.44 (0.18)	1.67 (0.31)	0.48 (0.12)
	PRX 0.3	31	258.13 (28.39)	0.42 (0.14)	0.92 (0.18)	0.47 (0.24)	2.19 (0.46)	0.85 (0.19)
	PRX 1.0	13	313.42 (48.28)	0.62 (0.22)	0.69 (0.26)	0.81 (0.32)	1.50 (0.30)	0.54 (0.18)
Random	Vehicle	32	49.32 (15.75)	0.08 (0.05)	1.05 (0.12)	1.02 (0.22)	1.95 (0.33)	0.89 (0.19)
	Buspirone 0.1	29	21.09 (5.15)	0.43 (0.16)	0.95 (0.17)	0.98 (0.34)	3.34 (0.65)	0.34 (0.16)
	Buspirone 0.3	4	2.94 (2.44)	0.62 (0.37)	0 (0)	0.88 (0.52)	0.25 (0.25)	0 (0)
	Buspirone 1.0	5	12.95 (12.45)	0.20 (0.20)	0.80 (0.37)	0.90 (0.40)	1.50 (0.71)	0 (0)
	Buspirone 3.0	4	0.50 (0.50)	1.50 (0.50)	0.50 (0.50)	0 (0)	0 (0)	0 (0)
	PRX	32	24.98 (7.87)	0.14 (0.06)	1.42 (0.15)	0.88 (0.22)	3.12 (0.64)	0.83 (0.20)
	PRX 0.1	29	43.13 (13.44)	0.10 (0.06)	1.18 (0.17)	1.18 (0.27)	2.12 (0.45)	0.80 (0.19)
	PRX 0.3	31	41.90 (12.34)	0.23 (0.09)	1.12 (0.20)	1.20 (0.07)	1.65 (0.44)	0.88 (0.26)
	PRX 1.0	9	90.67 (40.31)	0.28 (0.19)	0.89 (0.22)	0.49 (0.01)	2.22 (0.46)	0.50 (0.29)

3.5. Negative geotaxis and side effects

A significant Line effect, $F(1,387)=4.46$, $p<0.05$, showed that High line pups took longer to turn 180° from head down to head up on the inclined plane than Random line pups, regardless of drug condition, Fig. 4a. A main effect of Drug condition, $F(8,387)=5.00$, $p<0.001$, indicated that buspirone at 0.3, 1.0 and 3.0 mg/kg significantly increased inclined latencies for all pups, $ps<0.01$, Fig. 4b. Weight was also a factor influencing pup latencies to turn 180°, $F(1,387)=3.896$, $p<0.05$. In addition, more High line pups consistently vocalized while on the inclined plane than Random line pups, $Chi^2\ (1)=16.12$, $p<0.001$, Fig. 4c. Inclined plane latencies were uncorrelated with the number of pups vocalizing on the inclined plane, however. Overall, a significant Drug effect, $Chi^2(8)=42.14$, $p<0.001$, indicated that doses of buspirone from 0.30 to 3.0 mg/kg decreased instances of inclined plane vocalization from vehicle levels in a dose-dependent fashion, $ps<0.001$. PRX-00023 did not affect the frequency of inclined plane vocalizations at any dose (Table 3).

High line pups took significantly less time than Random line pups to flip from a supine to a prone position in the righting reflex test, $F(1,387)=12.10$, $p<0.001$. A main effect of Drug, $F(8,387)$ 12.74, $p<0.01$, indicated that buspirone at 0.3 and 3.0 mg/kg significantly increased latencies to flip from supine to prone compared to vehicle, ($p=0.014$ and $p<0.001$, respectively), indicating sedative effects at these doses. A significant Line×Drug interaction, $F(8,387)=3.55$, $p<0.001$, showed that for Random line pups, buspirone at 0.3, and 3.0 mg/kg increased righting latencies, $ps<0.001$, whereas High line pups were only affected by buspirone at 3.0 mg/kg, $p<0.001$. Sex and weight of pups were also significant covariates, $F(1,387)=3.896$, $p<0.05$ and $F(1,387)=7.896$, $p<0.01$. The frequency of head-weaving in pups was significantly elevated by buspirone over vehicle levels at 0.3, 1.0 and 3.0 mg/kg, Drug condition, $F(8,301=6.406, p<0.01$. Head-weaving was also less frequent in High than in Random line pups, $F(1,301)=12.634$, $p<0.001$; and a Line×Drug effect, $F(1,301)=3.043$, $p<0.05$, showed that whereas High line pups were affected by buspirone only at 1.0 and 3.0 mg/kg ($ps<0.05$), Random line pups were affected by all doses beginning with 0.3 mg/kg, $ps<0.05$.

4. Discussion

In High line pups, all doses of buspirone and the lowest doses of PRX-00023 significantly reduced vocalizations below vehicle controls. Although buspirone reduced USVs to a greater extent than PRX-00023 in High line rats, PRX-00023 affected USV rates at the lowest doses (0.01–0.05 mg/kg), a result consistent with enhanced efficacy at 5-HT1A receptors to produce anxiolytic effects. Thus, USV rates in High line rats were as sensitive to extremely low doses of PRX-00023 as they were to the lowest dose (0.10 mg/kg) of buspirone.

In contrast, Random line pup vocalizations appeared to be relatively unaffected by either drug. Historically there is a great deal of evidence that Random line pups experience separation anxiety, to

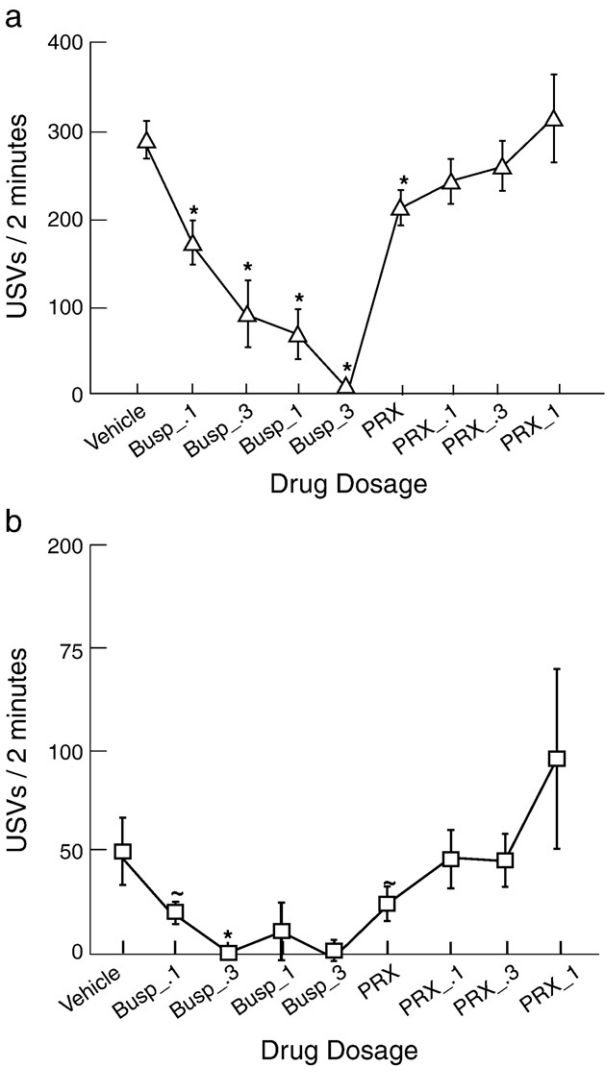

Fig. 3. Line and Drug effects on USV rates in isolation for 2 min by drug doses (PRX = PRX-00023; Busp = buspirone). * $p<0.05$, ~$p<0.10$. a. High line USV rates. b. Random line USV rates.

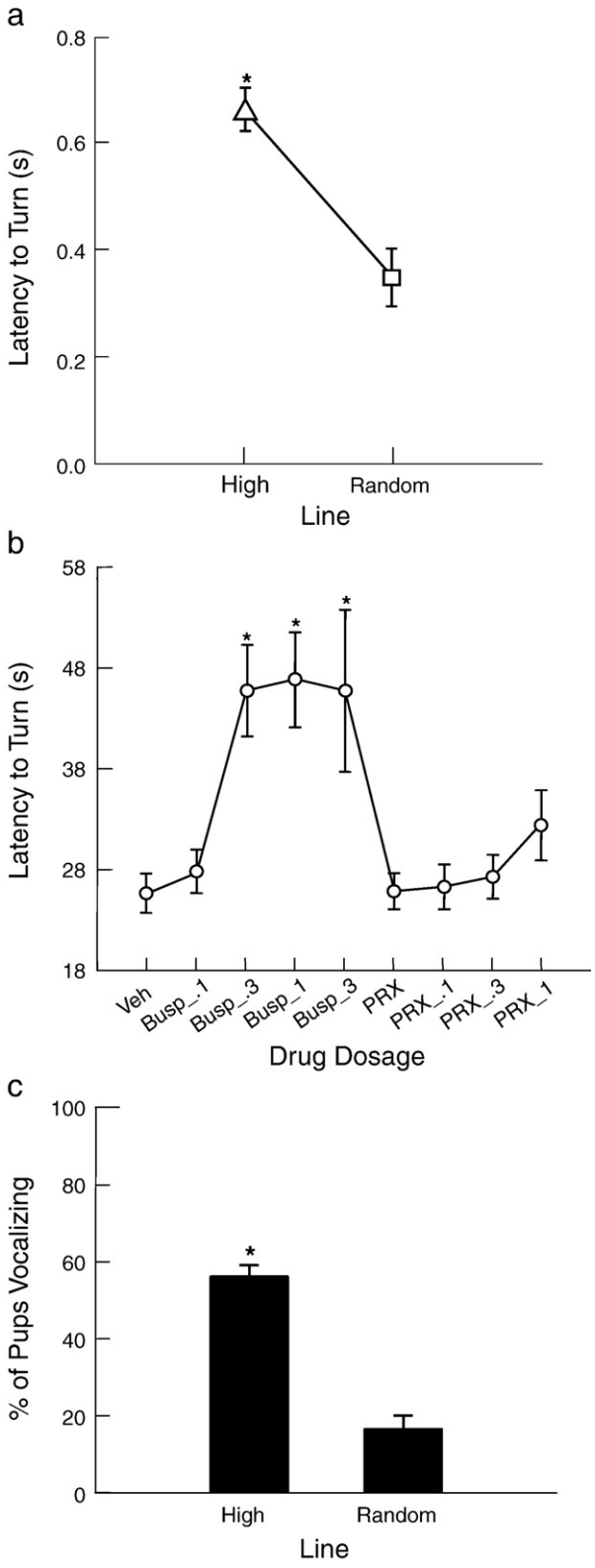

Fig. 4. Line and Drug effects on inclined plane. * $p<0.05$, ~$p<0.10$. a. High versus Random line latency to turn 180°. b. Drug and dose effects on latency to turn 180° (PRX=PRX-00023; Busp=buspirone; Veh=Vehicle). c. Percent of High versus Random vocalizing. Line differences in: * $p<0.05$, ~$p<0.10$.

judge from USV rates. In fact, Fig. 1 may be taken as evidence for both the existence of and variation in isolation-induced USV rates in Random line pups over 20 generations. Our laboratory has found that Random line pup rates of vocalization at 10–12 days of age are within the same range shown by a variety of rat strains (Brunelli et al., 1996; Brunelli and Hofer, 1996), and Random line pups show a normal developmental progression of isolation-induced USV rates from postnatal day 3 of life to postnatal day 21 compared to other strains (Brunelli and Hofer, 1996; Hofer et al., 2001). Random line pup isolation-induced USV can also be potentiated by brief exposure to the mother in isolation, and compared to isolation-induced USV these potentiated USVs have their own bout structure characteristics (Myers et al., 2004).

The most likely reason, then, for the apparent lack of anxiolytic activity of drugs in Random line pups was variation in their USV rates shown in this experiment. The high degree of variation in Random line pup calls is representative of the N:NIH strain base population on which the selective breeding was done, which makes them the obvious control for variation in the High and Low USV lines. In the current experiment variability in Random line USV rates in vehicle-treated animals from each litter (ranging from 0 to 413 USVs/2 min) would appear to have been great enough to observe significant reductions in USV with drug treatment. However, any given sample of Random line pups may not represent the normal distribution of USV rates in the N:NIH strain. In the current study the distribution was skewed so that a majority of pup USV rates were extremely low, with more than 50% of Random line pup rates under 20 calls per 2 min. Power calculations indicated that the numbers of subjects in several cells of the multivariate ANOVA were too small to detect significant effects, given the range of variation in Random line pups. However, when alpha restrictions were relaxed, *t*-tests revealed that the two lower doses of buspirone reduced vocalizations below vehicle levels in Random line pups, whereas PRX-00023 did not. We think this is a reasonable solution from which we may derive tentative conclusions about drug effects on Random line pups, provided they are followed by future tests with the requisite number of pups per drug group. These data are also consistent with studies showing that doses of buspirone at 0.1 mg/kg and higher effectively reduce parameters of USV in isolated rat pups (Hodgson et al., 2008; Olivier et al., 1998). It may therefore be provisionally concluded that High line pups are more sensitive to the anxiolytic properties of the 5-HT1A agonists than Random line pups.

With age, weight and sex accounted for in analyses, the lines did not differ on several measures of activity: rises, squares crossed in the cage and turning in square. Random line pups tended to face wash more than High line pups, however. In adult rats, face washing is considered a "displacement activity", as an animal is coping with two competing states (e.g., a state in which behavior is inhibited versus a state in which exploratory behavior may take place; Tinbergen, 1953). Turns-in-square were reduced below vehicle levels at doses of 0.1 to 1.0 mg/kg buspirone in both High and Random line pups, but were unaffected by any dose of PRX-00023. In 5-HT1A knockout mouse pups turns-in-square appear to be related to the broadcast of USVs, suggesting that this behavior and USV are modulated by activity of the 5-HT1A receptor (Ramboz et al., 1998). It is unclear whether the reduction of turns by buspirone were 5-HT-mediated and/or related to anxiety, or if the reductions were a secondary effect of sedation. We have never noted differences between High and Random or Low lines in this behavior over generations of selection, nor was it affected by PRX-00023, suggesting that turn-in-square is not specific to 5-HT1A receptor action in rats.

Beginning in the 11th generation of selection, analyses have shown that High line pups defecate more than both Random and Low line pups (Brunelli, 2005); however, these analyses typically involve hundreds of pups and litters. Since defecation in isolation is a low frequency behavior, there may have been insufficient power in the present study to detect High-Random differences compared to larger *n*'s used in previous studies. In adult rats, factor analyses have revealed that defecation usually loads on a separate factor from behavioral

Table 3
Side effect profiles: mean (sem).

Line	Drug condition mg/kg	Sample *n*	Head-weave frequency	Inclined plane latency (s)	Inclined plane vocalization % pups	Righting latency (s)
High	Vehicle	45	0 (0)	33.13 (2.85)	94	0.63 (0.03)
	Buspirone 0.1	40	0.02 (0.02)	30.70 (3.02)	69	0.62 (0.04)
	Buspirone 0.3	14	0.14 (0.10)	44.18 (5.08)	36	0.73 (0.11)
	Buspirone 1.0	16	0.29 (0.11)	46.16 (4.83)	39	0.86 (0.12)
	Buspirone 3.0	4	0.25 (0.25)	60.00 (0)	0	2.37 (0.94)
	PRX	39	0.03 (0.03)	33.53 (3.00)	82	0.80 (0.23)
	PRX 0.1	32	0 (0)	29.80 (3.86)	89	0.61 (0.04)
	PRX 0.3	31	0 (0)	32.97 (3.70)	90	0.68 (0.06)
	PRX 1.0	13	0 (0)	37.19 (6.09)	82	0.55 (0.04)
Random	Vehicle	32	0.05 (0.04)	18.75 (2.47)	55	0.68 (0.08)
	Buspirone 0.1	29	0.08 (0.05)	24.26 (3.13)	54	0.76 (0.09)
	Buspirone 0.3	4	0.50 (0.20)	53.63 (6.37)	0	2.12 (0.68)
	Buspirone 1.0	5	0 (0)	54.90 (5.10)	0	1.10 (0.37)
	Buspirone 3.0	4	1.00 (0)	36.00 (3.00)	0	2.50 (1.50)
	PRX	32	0.17 (0.07)	16.28 (2.28)	44	0.75 (0.11)
	PRX 0.1	29	0.15 (0.07)	21.50 (3.21)	61	0.61 (0.07)
	PRX 0.3	31	0.07 (0.05)	21.27 (2.94)	60	0.67 (0.07)
	PRX 1.0	9	0 (0)	30.11 (8.02)	61	0.49 (0.01)

measures of anxiety. This "autonomic" factor is commonly labeled as a component of known central–autonomic correlates of fear and anxiety (Fernandez-Teruel et al., 2002; Henderson et al., 2004). Although defecation in rat pups is consistent with this explanation, paradoxical increases in defecation by buspirone at 3.0 mg/kg are also consistent with findings that high doses (mM) of buspirone injected into adult rat hippocampus act to increase defecation in adult rats (Croci et al., 1995), though this has not been noted in peripheral administration.

There were significant differences in other side effect profiles of the two drugs. PRX-00023 did not affect body temperature at any dose tested. In contrast, buspirone reduced temperatures in a dose-dependent fashion. Olivier et al. (1998) reported comparable temperature effects at 1.0 and 3.0 mg/kg in 9–11 day-old Harlan/CPB strain rat pups. In adult animals hypothermia and sedation have been attributed to buspirone's affinity for mesocortical DA transmission, via postsynaptic 5-HT1A receptor pathways (Eltayb et al., 2001; Protais et al., 1998; Sakaue et al., 2000; Suaudeau et al., 1995); this also appears to be true in rat pups (Shayit et al., 2003). However, because PRX-00023 is more "purely" a 5-HT1A receptor agonist it would be less likely to enlist post-synaptic DA receptors as do more conventional 5-HT1A agonists such as the azapirones (Becker et al., 2006).

High line pups as a group had longer latencies than Random line pups to turn 180° from head down to head up on the inclined plane. The sedative effects of buspirone were also clearly observed in increased latencies to turn for both lines on inclined plane, and are consistent with previous reports (Hodgson et al., 2008; Olivier et al., 1998); this was not the case for any dose of PRX-00023. Most High line pups consistently vocalized on the inclined plane, although this behavior was not correlated with inclined plane latencies. On the other hand, High line pups were less sensitive to buspirone's sedative effects than Random line pups, as measured by righting latencies. Buspirone also produced significantly more head-weaving in both lines at several doses. Moreover, high line pups displayed less head-weaving than Random line pups; these, together with reduced latencies for righting, suggest possible reduced sensitivity to buspirones' postsynaptic effects (Bortolozzi et al., 1999; Jackson and Kitchen, 1989; Shayit et al., 2003). During the perinatal period these reflexes are altered by up-regulation of the 5-HT system in rats, at a time when 5-HT is acting as a neurotrophin (Bortolozzi et al., 1999).

Overall, USV rates in High line pups were responsive to the effects of PRX-00023 at doses that were orders of magnitude lower than its anxiolytic effects for adult animals (De Paulis, 2007). Moreover, the difference in the degree to which PRX-00023 reduced USV rates in High versus Random line pups suggests greater involvement of 5-HT1A receptors in High line pup USV rates. It is not known, however, whether these effects are due to pre- or postsynaptic actions of the 5-HT1A receptor. Based on human and animal studies the consensus at present is that both pre- and post-synaptic 5-HT1A receptors contribute to anxiety and/or depression, with overall serotonergic tone as a background that determines the relative strength of 5-HT1A receptor activity (Barnes and Sharp, 1999; Blier et al., 1997; De Vry, 1995; Millan, 2003).

One limitation of the current study is that the agonist effects of the 5-HT1A agonists buspirone and PRX-00023 were not challenged by the serotonin-1A receptor antagonist WAY100, 635 to establish specificity of its action at 5-HT1A receptors in rat pups. Because activity of underlying neural circuitry is still developing postnatally (Daval et al., 1987; Gallineau et al., 2004; Patel and Zhou, 2005), it is possible that agonist–antagonist actions at the 5-HT1A receptor in infant rats do not parallel those in adults. Moreover, antagonist studies might be able to further establish mechanisms by PRX-00023 seems to be effective at reducing USV rates in High line pups but not in Random line pups. Further studies are planned to determine this in High and Random line rat pups.

Despite its limitations, this study has revealed the potency of the novel 5-HT1A agonist PRX-00023, to reduce ultrasonic vocalizations as an expression of infantile anxiety at lower doses than buspirone. Moreover, PRX-00023 reduces this anxiety-like behavior at doses orders of magnitude lower than in adult rats, indicating a major developmental discontinuity in its potency. PRX-00023 also appears to have revealed inherent differences in 5-HT1A modulation in a genetic animal model of inherited early anxiety and adult depression.

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References

Allgulander C, Bandelow B, Hollander E, Montgomery SA, Nutt DJ, Okasha A, et al, World Council of Anxiety. WCA recommendations for the long-term treatment of generalized anxiety disorder. CNS Spectr 2003;8(8 Suppl 1):53–61.
Baldwin DS, Polkinghorn C. Evidence-based pharmacotherapy of generalized anxiety disorder. Int J Neuropsychopharmacol 2005;8(2):293–302.
Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacol 1999;38:1083–152.
Becker O, Dhanoa D, Marantz Y, Chen D, Shacham S, Cheruku S, et al. An integrated in silico model-driven discovery of a novel, potent, and selective amidosulfonamide 5-HT1A agonist (PRX-00023) for treatment of anxiety and depression. J Med Chem 2006;49:3116–35.
Blier P, Bergeron R, Montigny C. Selective activation of postsynaptic 5-HT1A induces rapid antidepressant response. Neuropsychopharmacol 1997;16(5):333–8.

Blumberg MS, Effimova IV, Alberts JA. Ultrasonic vocalizations by rat pups: the primary importance of ambient temperature and thermal significance of contact comfort. Dev Psychobiol 1992;25(4):229–50.
Blumberg MS, Knoot TJ, Kirby RF. Neural and hormonal control of arterial pressure during cold exposure in unanesthetized week-old rats. Am J Physiol Regul Integr Comp Physiol 2001;281(5):R1514–21.
Bortolozzi AA, Duffard RO, Evangelista de Duffard AM. Behavioral alterations induced in rats by a pre- and postnatal exposure to 2,4-dichrophenoxyacetic acid. Neurotoxicol Teratol. 1999;21(4):451–65.
Brunelli SA. Selective breeding for an infant phenotype: rat pup ultrasonic vocalization (USV). Behav Genet 2005;35(1):53–65.
Brunelli, S.A., Hofer, M.A. Selective breeding for an infantile phenotype (isolation calling): A window on developmental processes. In Blass, E.M. (Ed) Developmental Psychobiology, vol. 13, Handbook of Behavioral Neurobiology, Adler (Ed) 2001; New York: Kluwer Academic/Plenum Publishers, pp. 433–82.
Brunelli SA, Keating CC, Hamilton NA, Hofer MA. Development of ultrasonic vocalization responses in genetically heterogeneous National Institute of health (N:NIH) rats. I. Influence of age, testing experience, and associated factors. Dev Psychobiol 1996;29(6): 507–16.
Brunelli SA, Hofer MA. Development of ultrasonic vocalization responses in genetically heterogeneous National Institute of Health (N:NIH) rats. II. Associations among variables and behaviors. Dev Psychobiol 1996;29(6):517–28.
Brunelli SA, Hofer MA. Selective breeding for infant rat separation-induced ultrasonic vocalizations: developmental precursors of passive and active coping styles. Behav Brain Res 2007;182(2):193–207 [Sep 4].
Brunelli SA, Vinocur DD, Soo-Hoo D, Hofer M. Five generations of selective breeding for ultrasonic vocalization (USV) Responses in N:NIH strain rats. Dev Psychbiol 1997;31:255–65.
Brunelli SA, Masmela JR, Shair HN, Hofer MA. Effects of biparental rearing on ultrasonic vocalization (USV) responses in rat pups (*Rattus norvegicus*). J Comp Psychol 1998;12(4):331–43.
Croci T, Landi M, Bianchetti A, Manara L. Drug-induced defaecation in rats: role of central 5-HT1A receptors. Br J Pharmacol 1995;115(1):203–9.
Darmani NA, Ahmad B. Long-term sequential determination of behavioral ontogeny of 5-HT1A and 5-HT1 receptor functions in the rat. J Pharmacol Expt Therapeut 1999;288:247–53.
Daval G, Verge D, Becerril A, Gozian H, Spampinato U, Hamon M. Transient expression of 5-HT1A receptor binding sites in some areas of the rat CNS during postnatal development. Int J Dev Neurosci 1987;5(3):171–80.
De Paulis T. Drug evaluation: PRX-00023, a selective 5-HT1A receptor for depression. Curr Op Invest Drugs 2007;8(1):78–86.
De Vry J. 5-HT1A receptor agonists: recent developments and controversial issues. Psychopharmacol (Berl) 1995;121(1):1–26.
Eltayb A, Lindbloom S, Oerther S, Ahlenius S. Additive hypothermic effects of the 5-HT1A receptor agonist 8-OH-DPAT and the dopamine D2/3 receptor agonist 7- OH-DPAT in the rat. Acta Physiol Scand 2001;172:205–9.
Edwards D, Chugani D, Chugani H, Chehab J, Malian M, Aranda J. Pharmacokinetics of buspirone in autistic children. J Clin Pharmacol, 2006;46:508–14.
Fernandez-Teruel A, Escorihuela RM, Gray JA, Aguilar R, Gil L, Gimenez-Llort L, et al. A quantitative trait locus influencing anxiety in the laboratory rat. Genet Res 2002;12 (4):618–26.
Fish E, Sekinda M, Ferrari P, Dirks A, Miczek K. Distress vocalizations in maternally separated mouse pups: modulations via 5-HT_{1A}, 5-HT_{1B}, and $GABA_A$ receptors. Psychopharmacol (Berl) 2000;149:277–85.
Gallineau L, Kodas E, Guilloteau D, Vilar MP, Chalon S. Ontogeny of the dopamine and serotonin transporters in the rat brain: an autoradiography study. Neurosci Lett 2004;363:266–71.
Hammad T. Results of the analysis of suicidality in pediatric trials of newer antidepressants. Presented at the FDA Joint Psychopharmacologic Drugs Advisory Committee and the Pediatric Advisory Committee Meeting; 2004. September 13–14, Washington, DC.
Hammad TA, Laughren T, Racoosin J. Suicidality in pediatric patients treated with antidepressant drugs. Arch Gen Psychiatry 2006;63(3):332–9.
Henderson ND, Turri MG, DeFries JC, Flint J. QTL analysis of multiple behavioral measures of anxiety in mice. Behav Genet 2004;34:267–93.
Hidalgo RB, Tupler LA, Davidson JRT. An effect-size analysis of pharmacologic treatments for generalized anxiety disorder. J Psychopharmacol 2007;21:864–72.
Hodgson RA, Guthrie DH, Varty GB. Duration of ultrasonic vocalizations in the isolated rat pup as a behavioral measure: sensitivity to anxiolytic and antidepressant drugs. Pharmacol Biochem Behav 2008;88(3):341–8.
Hofer MA, Shair HN. Ultrasonic vocalizations during social interaction and isolation in 2-week-old rats. Dev Psychobiol 1978;11(5):495–504.
Hofer MA, Shair HN. Ultrasonic vocalization, laryngeal braking and thermogenesis in rat pups: a reappraisal. Behav Neurosci 1993;107(2):354–62.
Hofer MA, Masmela JR, Shair HN, Brunelli SA. Developmental effects of selective breeding for an infantile trait: the rat pup ultrasonic isolation call. Dev Psychobiol 2001;39:231–46.
Iyer GR, Reinhard Jr JF, Oshana S, Kauffman M, Donahue S. Tolerability, pharmacokinetics, and neuroendocrine effects of PRX-00023, a novel 5-HT1A agonist, in healthy subjects. J Clin Pharmacol 2007;47:817–24.
Jackson HC, Kitchen I. Behavioural profiles of putative 5hydroxytryptamine receptor agonists and antagonists in developing rats. Neuropsychopharmacology 1989;28(6):635–42.
Kaizar EE, Greenhouse JB, Seltman H, Kelleherc K. Do antidepressants cause suicidality in children? A Bayesian meta-analysis. Clin Trials 2006;3:73–98.
Kehne J, McCloskey T, Baron B, Chi E, Harrison B, Whitten J, et al. NMDA receptor complex antagonists have potential anxiolytic effects as measured with separation-induced ultrasonic vocalizations. Eur J Pharmacol 1991;193:283–92.
Leonardo ED, Hen R. Anxiety as a developmental disorder. Neuropsychopharmacol 2008;33(1):134–40 [Jan].
Millan MJ. The neurobiology and control of anxious states. Prog Neurobiol 2003;70:83–244.
Mos J, Olivier B. 1989. Ultrasonic vocalizations by rat pups as an animal model for anxiolytic activity: effects of serotonergic drugs. In: Bevan P, Cools AR, Archer T, editors. Behavioural pharmacology of 5-HT. Hillsdale, N.J.: Lawrence Erlbaum.
Murphy TK, Segarra A, Storch EA, Goodman WK. SSRI adverse events: how to monitor and manage. Int Rev Psychiatry 2008;20(2):203–8.
Myers MM, Ali N, Weller A, Brunelli SA, Tu AY, Hofer MA, et al. Brief maternal interaction increases number, duration, amplitude, and bout length of isolation-induced ultrasonic vocalizations in infant rats. J Comp Psychol 2004;118:95–102.
Olfson M, Shaffer D, Marcus SC, Greenberg T. Relationship between antidepressant medication treatment and suicide in adolescents. Arch Gen Psychiatry 2003;60 (10):978–82.
Olivier B, Molewijk HE, van der Heyden JAM, van Oorschot R, Ronken E, Mos J, et al. Ultrasonic vocalizations in rat pups: effects of serotonergic ligands. Neurosci Biobehav Rev 1998;23:215–27.
Patel TD, Zhou FC. Ontogeny of 5-HT1A receptor expression in the developing hippocampus. Dev Brain Res 2005;157:42–57.
Protais P, Lesourd M, Comoy E. Similar pharmacological properties of 8-OH-DPAT and alnesprione (S20499) at dopamine receptors: comparison with buspirone. Eur J Pharmacol 1998;352:179–87.
Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendeelshohn M, Mann JJ, Brunner D, Hen R. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci USA. 1998;95(26):1513–4.
Rickels K, Mathew S, Banoy MD, Zunbroff DL, Oshana S, Parsons Jr EC. Effects of PRX-00023, a novel, selective serotonin 1A receptor agonist on measures of anxiety and depression in generalized anxiety disorder: results of a double-blind, placebo-controlled trial. J Clin Psychopharmacol 2008;28(2):235–9.
Sakaue M, Somboonthum P, Nishihara B, Koyama Y, Hashimoto H, Baba A, et al. Postsynaptic 5-hydroxytryptamine(1A) receptor activation increases in vivo dopamine release in rat prefrontal cortex. Br J Pharmacol 2000;129(5):1028–34.
Salazar DE, Frackiewicz EJ, Dockens R, Kollia G, Fulmor IE, Tigel PD. Pharmacokinetics and tolerability of buspirone during oral administration to children and adolescents with anxiety disorder and normal healthy adults. J Clin Pharmacol 2001;41:1351–8.
Schwartz C, Snidman N, Kagan J. Adolescent social anxiety as an outcome of inhibited temperament in childhood. J Am Acad Child Adolesc Psych 1999;38(8):1008–15.
Shair HN, Jasper A. Decreased venous return is neither sufficient nor necessary to elicit ultrasonic vocalizations from rat pups. Behav Neurosci 2003;117(4):840–53.
Shayit M, Yadid G, Overstreet DH, Weller A. 5-HT1A receptor subsensitivity in infancy and supersensitivity in adulthood in an animal model of depression. Brain Res 2003;980:100–8.
Suaudeau C, Dourmap N, Costentin J. Rapid and long lasting reduction of the hypothermic effect of a D2 dopamine agonist after an intracerebroventricular injection of 6-hydroxydopamine. Neuropharmacol 1995;1:101–5.
Tinbergen N. Social Behavior in Animals. London: Methuen; 1953.
Weissman M, Warner V, Wickramarantne P, Moreau D, Olfson M. Offspring of depressed parents: 10 years later. Arch Gen Psychiatry 1997;54:932–40.
Weissman M, Wickramaratne P, Nomura Y, Warner V, Pilowsky D, Verdeli H. Offspring of depressed parents: 20 years later. Am J Psych 2006;163(6):1001–8.
Wiedenmayer CP, Barr GA. Developmental changes in responsivity to threat are stimulus-specific in rats. Dev Psychobiol 2001;39(1):1–7.
Wilkinson L. Systat 7.0: Statistics. Chicago: SPSS, Inc; 1997.
Zimmerberg B, Brunelli S, Fluty A, Frye C. Differences in affective behaviors and hippocampal allopregnanolone levels in adult rats of lines selectively bred for infantile vocalizations. Behav Brain Res 2005;159:301–11.
Zorrilla EP. Multiparous species present problems (and possibilities) to developmentalists. Dev Psychobiol 1997;30:141–50.
Zuckerman ML, Vaughan BL, Whitney J, Dodds A, Yakhkind A, MacMillan C, et al. Tolerability of selective serotonin reuptake inhibitors in thirty-nine children under age seven: a retrospective chart review. J Child Adolesc Psychopharm 2007;17(2):65–74.