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Rats Selectively Bred for Low Levels of 50 kHz Ultrasonic Vocalizations Exhibit Alterations in Early Social Motivation

ABSTRACT: *In rats, the rates of 50 kHz ultrasonic vocalizations (USVs) can be used as a selective breeding phenotype and variations in this phenotype can be an indicator of affective states. The 50 kHz USV is elicited by rewarding stimuli (e.g., food, sexual behavior) and therefore can express a positive affective state. Conversely, the 22 kHz USV is elicited by aversive stimuli (e.g., presence of a predator, social defeat) indicating a negative affective state. In the present study, we tested the effect of selectively breeding for 50 kHz USVs on a variety of maternal social/emotional behaviors in young rat pups (PND 10-12). These measures consisted of an assessment of isolation calls and conditioned odor preference paradigm. Results indicate that animals selected for low levels of 50 kHz USVs show the greatest alterations in social behaviors compared to the control animals. The low line animals had an increase in isolation calls tested during place preference conditioning and a decrease in 50 kHz ultrasonic calls in all conditions. These same low line animals failed to show a typical preference for a maternally-associated odor during the place preference test. The different social behaviors of the high line animals did not consistently vary from those of the control group. These results have important implications for the study of genetic and epigenetic mechanisms underlying emotional states, and possibly contribute to the research underlying the emotional changes in developmental disorders such as autistic spectrum disorder by providing a novel animal model that displays communication deficits that are interdependent with significant social behavioral impairments.*
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INTRODUCTION

The study of emotional vocalizations in mammals is becoming one of the major ways to access the social–emotional circuitries of the brain. For instance, Panksepp and colleagues have examined the neuroanatomical and neurochemical controls of isolation vocalizations of young dogs, guinea pigs and domestic chicks, and have found remarkable similarities in the controlling variables of vocalizing among these animal groups (Nelson & Panksepp, 1998; Panksepp, Herman, Vilberg, Bishop, & DeEskinazi, 1980; Panksepp, Normansell, Herman, Bishop, & Crepeau, 1988; also see Panksepp, 1998 for review). The neuroscience of emotion using similar methods has extended to various other species, including primates (Kalin, Shelton, & Barksdale, 1988; Newman, 1988, 1991) and infant rodents (Costantini & D'Amato, 2006). In general, these findings have increased significantly the understanding of human emotions such as sadness and grief that can follow separation or loss (Panksepp, 2003).

During the past 4 years, we have selectively bred rats of the Long-Evans strain for low or high levels of 50 kHz ultrasonic vocalizations (USVs) (Burgdorf, Panksepp,

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Brudzynski, Kroes, & Moskal, 2005) in order to segregate the alleles that are responsible for this indicator of apparent positive social affect (Burgdorf & Panksepp, 2006; Knutson, Burgdorf, & Panksepp, 2002; Panksepp & Burgdorf, 2003). We are currently in the 17th generation of selection. Our aim is to select for this emotional vocalization and produce animals that differ in their social-emotional responsivity. If social motivation is related to central integrator mechanisms that regulate emission of these calls, then we might anticipate animals that have constitutional differences in the emission of 50 kHz USVs to exhibit differences in social motivation. For instance, if animals are less capable of exhibiting positive social affect, as indexed by 50 kHz USVs, we might anticipate that they will exhibit stronger emotional-vocal responses to social separation. Likewise, they may exhibit differential social bonding to maternal cues, perhaps exhibiting a decreased ability to bond since they may have decreased positive social affect. The experiments summarized in this paper were designed to evaluate such possibilities.

Previous work has highlighted that the rates of 50 kHz USVs have social meaning for rats. For instance, rats that display high rates of 50 kHz USVs during heterospecific hand-play (henceforth ''tickling'') demonstrate more play behaviors than animals that exhibit low levels of 50 kHz USVs (Panksepp, Gordon, & Burgdorf, 2002). Situations involving rewarding stimuli such as food, sexual partners, play opportunities, rewarding drugs of abuse, rewarding electrical stimulation of the lateral hypothalamus, and social contact following acute isolation all elicit 50 kHz vocalizations, suggesting that this vocalization may be used as a general measure of positive reward motivation, especially social motivation (Brudzynski & Pniak, 2002; Burgdorf & Panksepp, 2006; Panksepp, Knutson, & Burgdorf, 2002), and from this it might be anticipated that one would observe reciprocal effects in animals that exhibit low-levels of these positive vocalizations. Namely animals bred for low levels of 50 kHz USVs might be expected to be more responsive to social isolation.

The analysis of USVs in rats has revealed that they generally exhibit three different types of USVs based on sound frequency and duration (Brudzynski, Bihara, Ociepa, & Fu, 1993; Miczek, Tornatzky, & Vivian, 1991) and each USV seems to have a specific context dependency. The first is a 40 kHz USV that typically occurs during infancy in response to isolation from the dam and littermates (Miczek et al., 1991; Sales & Pye, 1974), commonly referred to as the isolation call or distress vocalization. The authors of this paper prefer the term isolation call. The second type of USV is a 22 kHz USV emitted by juvenile and adult rats in response to exposure to predators (Blanchard, Blanchard, Agullana, & Weiss, 1991), exposure to pain (Tonoue, Ashida, Makino, & Hata, 1986), during intermale fighting (Thomas, Takahashi, & Barfield, 1983), and during the refractory period after copulation (Barfield and Geyer, 1972). The third type of USV is a 50 kHz USV that is emitted quite frequently in adult rats during social exploration (Blanchard, Yudko, Blanchard, & Taukulis, 1993; Brudzynski & Pniak, 2002), even more during proceptive sexual behavior (McGinnis & Vakulenko, 2003; McIntosh & Barfield, 1980), and even more abundantly in juvenile rats in response to solicitation of play (Knutson, Burgdorf, & Panksepp, 1998), with maximal levels seen during tickle-induce reward (Panksepp & Burgdorf, 1999, 2000). There are also some 50 kHz USVs during male agonistic behaviors during fighting (Sales & Pye, 1974), but these seem to occur exclusively prior to the onset of active fighting (Burgdorf, Panksepp, Kroes, & Moskal, 2007).

Isolation calls have been a very effective method of studying affective states in preweanling rats (Brunelli, Vinocur, Soo Hoo, & Hofer, 1997; Winslow et al., 2000) because eliciting isolation calls is reliably consistent. Such isolation vocalizations in various species have many neuropharmacological similarities to anxietylike behaviors in adulthood and anxiety as well as depressive disorders in humans (reviewed in Hofer, 1995, 1996; Miczek et al., 1991; Newman, 1991; Panksepp, Yates, Ikemoto, & Nelson, 1991; Winslow & Insel, 1991). Previous research has indicated that isolation calls are negatively correlated with positive affective states (Dichter, Brunelli, & Hofer, 1996; Vivian, Barros, Manitiu, & Miczek, 1997). This has also been demonstrated with neuropharmacological studies showing that benzodiazepines decrease isolation calls (Vivian et al., 1997) and also activity within an elevated-plus maze indicated that animals with a high rate of isolation calls as infants were less likely to spend time investigating during this test (Dichter et al., 1996). Thus, our prediction is that animals that are constitutionally emitting 50 kHz USVs infrequently should exhibit heightened rates of infant isolation calls. The two behavioral tests utilized in the present work include the well-studied isolation call test and the conditioned odor preference test using a maternal-associated odor cue. The latter measure borrows elements from the well-established conditioned place preference task (Bardo & Bevins, 2000; Tzschentke, 1998). A previous study by Nelson and Panksepp (1996) was used as a general template for evaluating differences in infant–mother bonding tendencies in our genetic lines. The aim of the present project is to see if animals presumptively different in positive affect vocalizations (50 kHz) expressed more social learning and prosocial behaviors than controls. This work was presented recently in preliminary form (Harmon et al., 2006).

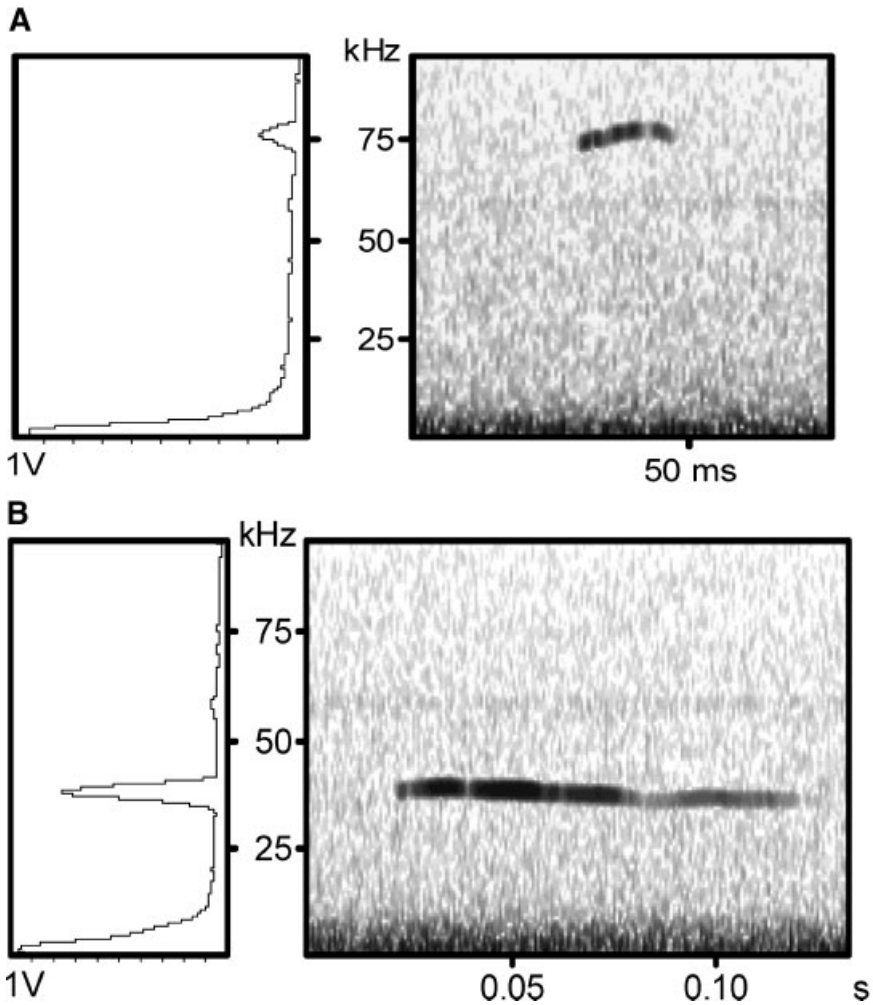

FIGURE 1 Sonogram of infantile USV. (A) Higher (>50 kHz) frequency call. (B) Classic isolation call. Graphs on the left hand side show power spectra of the relevant calls.

METHODS

Subjects

All breeding animals were Long-Evans rats originally purchased from Charles River (St. Constant, Quebec, Canada). All the selection work was conducted at Brock University Animals Facility (St. Catharine's, Ontario, Canada) where they were selectively bred for low, random and high genetic lines (see Burgdorf et al., 2005 for the original report on these animals, currently in their 17th generation of selection). At approximately 90 days of age, breeding pairs of the various lines (from the 13th generation of selection) were transferred to Bowling Green State University. Animals were mated at Animal Facilities at Bowling Green State University (Bowling Green, OH) with one male per female. For this study, only litters yielding 8 or more pups were used for testing. The animals were housed in clear plastic cages (65 cm × 24 cm × 15 cm) with food (Harlan Teklad Rat Chow #8604) and tap water ad libitum. Corn-cob chips were provided for bedding. Subjects were maintained on a day-night cycle of 12:12 light/dark cycle (lights on at 07:00) and room temperature was kept at 22°C and humidity was controlled at 40–50%. The Institutional Animal Care and Use Committee at Bowling Green State University approved all procedures (Bowling Green State University IACUC Protocol #04-013).

Isolation Calls

We examined the production of isolation calls between the genetic lines. The isolation testing chamber, which was located in a testing room separate from the housing room, consisted of a 500 ml glass beaker with an ultrasonic microphone suspended approximately 25 cm above the base of the beaker. USVs were recorded using a high frequency bat detector, Pettersson D980 ultrasonic detector (Uppsala, Sweden) that digitally recorded at 196 kHz, and USVs were analyzed offline via sonogram (Avisoft, Bioacoustics, Berlin, Germany). The pups were habituated for 1 min to the testing chamber on postnatal day 9. On postnatal day 10, the pups were removed from the colony room and were placed individually in the isolation testing

apparatus for 2 min with USVs being recorded. There were no other animals present in the testing room during the testing session. The pup body temperature was not monitored during the testing session, but the testing room was maintained at 22 ± 1°C. After testing, animals were transported back to the colony room and were returned to their home cage and dam. Animals were tested during the light cycle of a 12:12 light/dark cycle (lights on at 07:00). Data were manually scored offline for total number of isolation calls and for the total number of higher frequency (>50 kHz) vocalizations (Fig. 1).

Conditioned Odor Preference (COP)

COP paradigm was designed to measure the affinity of the pup to its dam. The testing apparatus consisted of a Plexiglas chamber (18 cm × 5 cm × 7.6 cm), which was divided crosswise by visual marker into three equal sections of 6 cm (Fig. 2). The three sections of the COP apparatus, described in Cromwell et al., 2007; Nelson & Panksepp, 1996) allow for a choice behavior to be displayed by the pups as well as allowing for a separation of the odor cue from the opposing side. This separation enhanced the choice behavior and discrimination between the two sides. The testing apparatus was located in a testing room, separate from the colony room. The chamber, which has metal bars across the floor, was placed directly on top two equally spaced open glass jars (~7.6 cm in height) which contained either a cotton ball saturated with 1 ml of pure lemon extract or a cotton ball saturated with 1 ml of distilled water.

Pups were removed from their home cage in the colony room and were habituated to the COP chamber on postnatal day 10 for 1 min. Conditioning began on postnatal day 11 and animals were separated into six conditioning groups: (1) high lines animals conditioned with cotton balls, (2) high line animals conditioned with the dam, (3) random line animals conditioned with cotton balls, (4) random line animals conditioned with the dam, (5) low line animals conditioned with cotton balls, and (6) low line animals conditioned with the dam.

Conditioning was conducted 3 times during postnatal day 11 with 3 hr of maternal deprivation preceding each 30 min conditioning session. Both conditioning and maternal deprivation time was performed in an experiment room other than the testing room or the colony room. Animals conditioned with the cotton ball were removed from their home cage and were placed in a novel cage (23.5 cm × 21 cm × 20.3 cm) with 1 cotton ball saturated with .25 ml of lemon extract on each cotton ball in each of the four corners. The groups conditioned with the dam were removed from their home cage and were placed in an identical novel cage with the dam that had been saturated with 1 ml of lemon extract on her ventral surface immediately prior to being placed in the novel conditioning cage. All animals remained in the conditioning cages for 30 min. Following the third conditioning session, the dam was bathed with soap and water to remove the lemon scent from her ventral surface and was returned, along with the pups, to the home cage.

Testing began the day immediately following the conditioning day (postnatal day 12). Testing was preceded by 3 hr of maternal deprivation with the pups housed as a litter. A testing session consisted of placing each pup in the middle of the testing apparatus and giving the pup free access to the place preference apparatus for 5 min. The side containing the lemon scent was counterbalanced across trials. Trials were recorded via commercially available DVD recorder and camera to be analyzed offline. USVs were also recorded during the COP task and analyzed by a blind experimenter with the same recording equipment and computer software used in the earlier social isolation paradigm. A trained experimenter, blind to testing conditions, used a computer-based behavioral scoring system to accurately code compartment entries and time duration via joystick control.

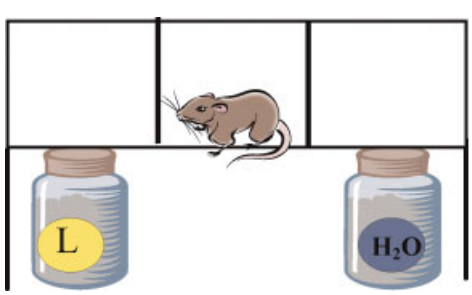

FIGURE 2 Schematic drawing of conditioned odor preference apparatus.

Statistical Analysis

For each dependent measure for the different behavioral paradigms, we performed a different analysis of variance (ANOVA). For each ANOVA, the independent factor was genetic line (high, random, and low). For COP data, addition factors were used that included the conditioning group (cotton or dam) and sex (male or female). Dependent measures for general litter characteristics included average litter size, average pup weight (postnatal day 10), and average litter weight (postnatal day 10). For COP testing, dependent measured included total number of line crosses, and a within subject variable for compartment location (scented or nonodor portion). Data are given as mean ± SEM. The litter mean was the unit of analysis for both the call and COP analysis. The threshold significance level for including the results reported in the text was $p < .05$. When this threshold criterion was met, post hoc *t*-tests were completed using a LSD correction for multiple comparisons. Data analysis was conducted with SPSS Software 13.0 and Microsoft Excel 2007.

RESULTS

Litter Statistics

There were seven high line litters, six random line litters, and five low line litters used, but only six high line and four low line litters were used for the isolation call testing and compartment entries due to technical difficulties (computer disk malfunction). For COP duration analysis, only five random line litters were used. The number of pups per litter was not statistically different among the high,

random and low groups ($F(2, 15) = .86$, $p = .4$; high line litter size: $12.2 \pm .9$; random line litter size: 13.6 ± 1.0; low line litter size: 13.8 ± 1.0). The average pup weight at postnatal day 10 differed statistically between the genetic lines ($F(2, 225) = 46.208$, $p < .001$; low line $= 22.0 \pm .3$ g; high line $= 19.4 \pm .4$ g; random line $= 18.0 \pm .3$ g). Pairwise comparisons showed that low line litters were significantly higher in weight compared to either random line ($t(151) = 10.4$, $p < .001$) or high line ($t(142) = 6.0$, $p < .001$). Additionally, the average litter weight at postnatal day 10 differed significantly ($F(16, 209) = 23.3$, $p < .001$; average low line $= 19.6 \pm .8$ g; average random line $= 18.2 \pm .9$ g; average low line $= 22.2 \pm .6$ g). Pairwise comparisons between average litter weights also showed that low line litters were significantly higher in weight compared to random line litters ($t(8) = 3.0$, $p < .05$) but not quite significantly heavier compared to the average high line litter ($t(8) = 2.1$, $p = .07$). Weight data are not all inclusive due to missing weight data.

Isolation Calls

There was a significant main effect between the genetic lines for the higher frequency (>50 kHz) calls ($F(2, 15) = 8.2$, $p < .01$; Fig. 3). However, there was no significant main effect between the genetic lines for isolation calls ($p = .4$). Post hoc analysis indicated that the low line animals and high line animals showed significantly fewer higher frequency (>50 kHz) calls compared to the random line (high line ($t(10) = 2.6$, $p < .05$; low line ($t(8) = 3.6$, $p < .01$).

Conditioned Odor Preference: Locomotion

For all COP analysis, gender never had a main effect or interaction with another variable, so it was therefore removed as a variable from this analysis. The total number of line crosses was examined to determine if either the high or low line animals showed increased locomotor activity. There was a significant main effect for locomotor activity ($F(2, 15) = 14.5$, $p < .001$; Fig. 4). The low line animals showed a significant increase in line crosses compared to the random line animals (low line: $t(8) = 3.0$, $p < .05$). The high line animals failed to show significant variations in line crosses compared to the random line animals ($p = .06$; Fig. 4).

Conditioned Odor Preference: Duration

There were no significant differences related to sex for main effects ($F(1, 54) = 1.27$, $p = .3$) There was a main effect for genetic line ($F(2, 28) = 5.183$, $p < .05$), which implied genetic lines, high, random and low lines had an effect on place preference. There was also a main effect for conditioning group (dam or cotton ball) ($F(1, 28) = 4.9$, $p < .05$). In addition, there was an interaction between place preference and genetic line ($F(2, 28) = 3.78$, $p < .05$; Fig. 5). Post hoc t-tests confirmed our hypothesis that random line animals conditioned with the dam showed a significant preference for the dam-associated scented compartment compared to the nonodor compartment ($t(4) = 3.6$, $p < .05$), while random animals conditioned with cotton balls fail to show a place preference ($t(4) = .798$, $p = .5$; Fig. 5). The high line animals conditioned with the cotton balls displayed

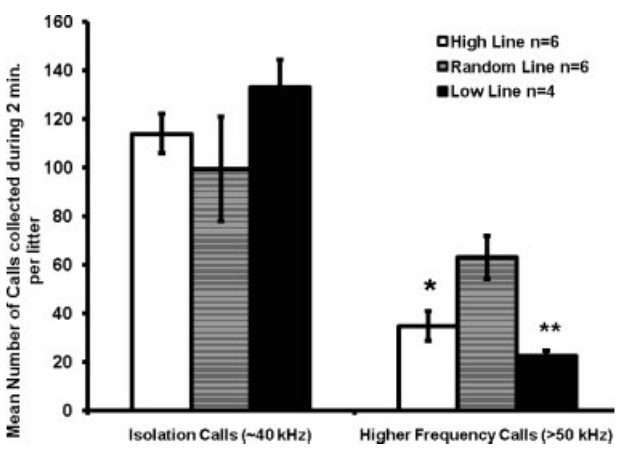

FIGURE 3 Mean number of USVs for each genetic line during the 2 min. isolation vocalization task. n indicates the number of litters. Significantly different compared to random line. $^{*}p < .05$ and $^{**}p < .01$.

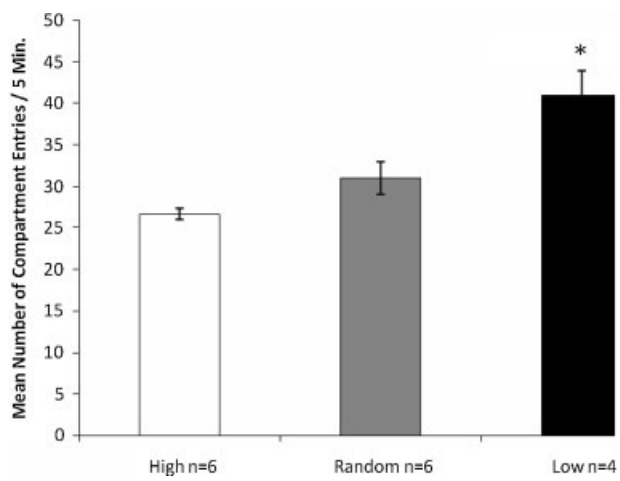

FIGURE 4 Combined mean number of line crosses into the lemon and into the water portion of testing apparatus in 5 min for each genetic line. Individual studentized t-tests analyzed high to random and low to random litter comparisons. $^{*}p < .05$.

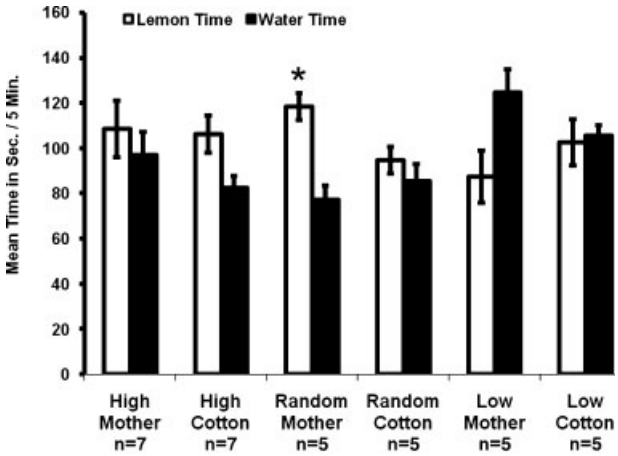

FIGURE 5 Mean amount of time in seconds per 5 min each genetic line, separated by conditioning group, spent on each side of the COP chamber. *n* indicates the number of litters. $^{*}p < .05$ lemon time compared to water time

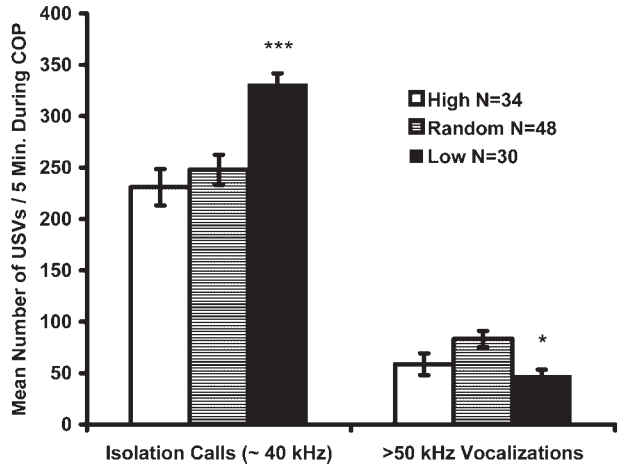

FIGURE 6 The mean number of >50 kHz distress and <50 kHz USVs for each genetic line during the COP testing. *n* indicates the number of pups for each line. $^{*}p < .05$ and $^{***}p < .001$ compared to random line.

preference for the scented portion regardless of conditioning group, but neither condition reached statistical significance. Finally, low line animals failed to show a significant preference based on conditioning group, but low line animals tend to prefer the nonscented portion the testing apparatus.

Conditioned Odor Preference: Vocalizations

Vocalizations were recorded and analyzed for a subsample of the animals (High line, $n = 34$; Low line $n = 30$; Random line, $n = 30$) during the COP test, to determine the rates of isolation calls and the rates of higher frequency (>50 kHz) calls for each genetic line (Fig. 6). Because data only include a subsample, litter means are not used as the unit of analysis; rather each pup sampled was a unit of analysis. As expected, the low line animals showed the most isolation calls compared to the random line ($t(76) = 4.08$, $p < .001$) and fewer higher frequency (>50 kHz) vocalizations ($t(76) = 3.24$, $p < .01$).

DISCUSSION

The results of the present study indicate that animals selected for low levels of 50 kHz USVs exhibit an increase in isolation calls when separated from their mother at two weeks of age. These same animals display an increase in their locomotor activity during the COP task, and most importantly, they lack the typical preference for a maternally-associated odor. In other related work, it has been shown that animals selected for low levels of 50 kHz USVs show significantly less social contact during a social interaction assay and produce more fecal boli during an open field task, indicating an increased negative emotionality (Burgdorf et al., 2007). These results are consistent with our hypothesis that animals selected for low levels of 50 kHz vocalizations have greater negative affectivity compared to randomly bred animals and could be a valuable animal model for mental illness characterized by elevated sadness and depression.

Our results complement work by Brunelli and colleagues showing that animals bred for greater isolation calls tend to exhibit elevated symptoms of anxiety later in life. They have shown that these differences in vocalizations that extend throughout different developmental stages (Brunelli, 2005; Brunelli, Keating, Hamilton, & Hofer, 1996) and they have identified other differential developmental markers in rats selected for the greater rates of isolation calls (Hofer, Shair, Masmela, & Brunelli, 2001). Their results demonstrate that animals that emit a higher level of isolation calls show more urination and defecation responses to 2 min of isolation and more pups with ear canals open at 10 days old compared to their random and low groups. In a similar way, the present work addressed social–functional relationships between expressions of high and low levels of 50 kHz USVs and other potential coselected traits.

Animals selected for elevated levels of 50 kHz calls do not differ from the random line animals with respect to isolation calls, raising question to the hypothesis that these animals show an increase in positive affectivity. Further testing during adulthood is needed to determine the effect of selecting for greater positive emotionality. Studies have already begun in this lab to assess gregariousness and

sociability of these selectively bred lines. However, Burgdorf et al. (2007) have shown that these high line animals exhibit greater sensitivity to sucrose reward and less anxiety and defensive aggression compared to random line animals. These data suggest positive affectivity differences specifically that these high line animals have an increase in positive affectivity that may be seen later in development. Continued behavioral testing is needed to confirm this hypothesis.

Each type of USV is emitted under a different environmental condition and the different subtypes have been proposed to indicate a different affective state. For example, the 22 kHz USV is elicited under threatening situations; it is a potential indicator of negative affect akin to anxiety evoked by a real or experimental aversive situation (Brudzynski, 2001; Miczek et al., 1991; van der Poel & Miczek, 1991). The infantile isolation vocalization is also elicited under aversive or threatening conditions such as maternal isolation and is thought to be an indicator of a negative affective state, even though this conclusion is controversial (Blumberg & Sokoloff, 2000; Hofer & Shair, 1993; Shair & Jasper, 2003; but see Panksepp, 2003 for response). It has been hypothesized that isolation calls seen in young rat pups are the precursor vocalization to the adult 22 kHz vocalization and that there is a linear function of vocalization frequency with larynx size (Blumberg & Alberts, 1991). Our recent preliminary developmental studies indicate that 22 kHz calls develop independently from isolation calls (Shields & Brudzynski, 2007). Higher frequency (>50 kHz) vocalizations seen in young rat pups could, however, develop in a different way. Perhaps these higher frequency vocalizations are the precursor to the adult 50 kHz vocalizations. However, these relationships have yet to be shown empirically. In any event, the 50 kHz USV is emitted during situations of potential reward, and researchers postulate that it is an indicator of positive emotional states similar to anticipatory excitement or eagerness (Knutson, Burgdorf, & Panksepp, 1999; Knutson et al., 2002).

The present study is also the first work to document a higher frequency (>50 kHz) vocalization during the infant isolation task. These calls are at a characteristic frequency vastly different than the isolation calls described in Brudzynski et al. (1993) and Brunelli (2005). Our sonographic analysis indicates a higher frequency call with a mean (±SEM) peak frequency at 66.4 ± 1.7 kHz and mean duration (±SEM) of 19.5 ± 3.8 ms, respectively. The traditional isolation calls seen here have a mean peak frequency (±SEM) and duration (±SEM) of 40.1 ± 1.0 kHz and 124.0 ± 17.9 ms, respectively, which is consistent with isolation calls described by previous researchers (Brudzynski, Kehoe, & Callahan, 1999). We are suggesting the possibility that pup isolation calls can be empirically divided into two categories, the first being the classic 40 kHz (peak frequency) call type and the latter being a much shorter and higher pitched call (~66 kHz peak frequency). Our data acquisition methods utilized high frequency recording that was able to monitor a broad spectrum of call types. Other more limited recording methods may not be sensitive enough to differentiate this range of calls. Further analysis and pharmacological manipulations are needed to ascertain the importance and significance of this finding.

In 1988, Panksepp was the first to show the strong inhibitory effect of oxytocin on isolation calls (Panksepp, 1988, 1992), implicating oxytocin in social-bonding processes above and beyond the instigation of maternal behavior. Accordingly, we also evaluated our animals in an oxytocin sensitive infant–mother social-bonding paradigm. The COP paradigm selected has been used to examine the influence of oxytocin on social attachment and learning: Preweanling rat pups were conditioned with either oxytocin, an oxytocin antagonist, or saline and were tested in the COP apparatus for conditioned approach and place preference using olfactory cues. Rat pups treated with either saline or oxytocin demonstrated a strong preference for the olfactory cue side of the apparatus whereas the oxytocin antagonist group failed to develop a strong preference for the maternally associated odor (Nelson and Panksepp, 1996). These findings are consistent with the extensive literature that brain oxytocin mediates many different prosocial behaviors including maternal behavior, sexual behavior, and social memory (Carter, 2003; Insel, 1997; Gammie, 2005; Nelson & Panksepp, 1998) and the role of oxytocin in regulating separation distress (Panksepp, 1988, 1992). Our results indicated that the lines bred for differential rates of 50 kHz calls behaved differentially in this oxytocin-sensitive task. Accordingly, in future studies it will be desirable to evaluate directly whether or not brain oxytocin levels differ in high and low 50 kHz selection lines.

These studies and others suggest that animals selectively bred for differential levels of 50 kHz USVs reflect variations in positive and negative affective phenotypes. Other studies evaluating behavioral variations between these animal groups during adolescence and adulthood have already highlighted other influences of the selection process throughout other portions of the lifespan (Burgdorf et al., 2007) which are generally consistent with these results. Current work using cellular and molecular techniques could reveal key genetic, and biochemical variations related to affective phenotypes. We anticipate this model may prove to be relevant for behavioral phenotypes seen in depression, autism and numerous other psychiatric disorders, as well as

contributing to our understanding of the heritability of affect and emotions.

NOTES

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REFERENCES

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward. Psychopharmacology, 153, 31–43.

Barfield, R. J., & Geyer, L. A. (1972). Sexual behavior: Ultrasonic postejaculatory song of the male rat. Science, 176, 1349–1350.

Blanchard, R. J., Blanchard, D. C., Agullana, R., & Weiss, S. M. (1991). Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. Physiology and Behavior, 50, 967–972.

Blanchard, R. J., Yudko, R. B., Blanchard, D. C., & Taukulis, H. K. (1993). High-frequency (35–70 kHz) ultrasonic vocalizations in rats confronted with anesthetized conspecifics: Effects of gepirone ethanol, and diazepam. Pharmacology, Biochemistry and Behavior, 44, 313–319.

Blumberg, M. S., & Alberts, J. R. (1991). On the significance of similarities between ultrasonic vocalizations of infant and adult rats. Neuroscience & Biobehavioral Reviews, 15, 383–390.

Blumberg, M. S., & Sokoloff, G. (2000). Do infants cry? Psychological Review, 108, 83–95.

Brudzynski, S. M. (2001). Pharmacological and behavioral characteristics of 22 kHz alarm calls in rats. Neuroscience Biobehavioral Review, 25, 611–617.

Brudzynski, S. M., Bihara, F., Ociepa, D., & Fu, X. (1993). Analysis of 22 kHz ultrasonic vocalization in laboratory rats: Long and short calls. Physiology and Behavior, 54, 215–221.

Brudzynski, S. M., Kehoe, P., & Callahan, M. (1999). Sonographic structure of isolation-induced ultrasonic calls of rat pups. Developmental Psychobiology, 34, 195–204.

Brudzynski, S. M., & Pniak, A. (2002). Social contacts and production of 50 kHz short ultrasonic calls in adult rats. Journal of Comparative Psychology, 116, 73–82.

Brunelli, S. A. (2005). Selective breeding for an infant phenotype: Rat pup ultrasonic vocalization (USV). Behavior Genetics, 35, 53–65.

Brunelli, S. A., Keating, C. C., Hamilton, N. A., & Hofer, M. A. (1996). Development of ultrasonic vocalization responses in genetically heterogeneous National Institute of Health (N: NIH) rats. I. Influence of age, testing experience, and associated factors. Developmental Psychobiology, 29, 507–516.

Brunelli, S. A., Vinocur, D. D., Soo Hoo, D., & Hofer, M. A. (1997). Five Generations of selective breeding for ultrasonic vocalization (USV) responses in N: NIH strain rats. Developmental Psychobiology 31, 255–265.

Burgdorf, J., & Panksepp, J. (2006). The neurobiology of positive emotions. Neuroscience and Biobehavioral Reviews, 30, 173–187.

Burgdorf, J., Panksepp, J., Brudzynski, S. M., Kroes, R., & Moskal, J. R. (2005). Breeding for 50-kHz positive affective vocalization in rats. Behavior Genetics, 35, 67–72.

Burgdorf, J., Panksepp, J., Kroes, R. A., & Moskal, J. R. (2007). The effect of selective breeding for differential rates of 50-kHz ultrasonic vocalizations on emotional behavior in rats. Developmental Psychobiology, (in press).

Carter, C. S. (2003). Developmental consequences of oxytocin. Physiology & Behavior, 79, 383–397.

Cromwell, H. C., Johnson, A., McKnight, L., Horinek, M., Asbrock, C., Burt, S., Jolous-Jamsjidi, C., & Meserve, L. A. (2007). Effects of polychlorinated biphenyls on maternal odor conditioning in rat pups. Physiology & Behavior, 91, 658–666.

Costantini, F., & D'Amato, F. R. (2006). Ultrasonic vocalizations in mice and rats: Social contexts and functions. Acta Zoologica Sinica, 52, 619–633.

Dichter, G. S., Brunelli, S. A., & Hofer, M. A. (1996). Elevated plus-maze behavior in adult offspring of selectively bred rats. Physiology and Behavior, 60, 299–304.

Gammie, S. C. (2005). Current models and future directions for understanding the neural circuitries of maternal behaviors in rodents. Behavior and Cognitive Neuroscience Reviews, 4, 119–135.

Harmon, K. M., Burgdorf, J., Moskal, J. R., Kroes, R. A., Panksepp, J., & Cromwell, H. C. (2006). Behavioral phenotyping of rats selectively bred for differential levels of 50 kHz ultrasonic vocalizations. Thirty-Sixth Annual Meeting of the Society for Neuroscience, Atlanta, Georgia, Neuroscience Meeting Planner, Abstract 578.10.

Hofer, M. A. (1995). An evolutionary perspective on anxiety. In: S. P. Roose, & R. A. Glick (Eds.), Anxiety as symptom and signal (pp. 17–38). London: Analytic Press.

Hofer, M. A. (1996). Multiple regulators of ultrasonic vocalization in the infant rat. Psychoneuroendocrinology, 21, 203–217.

Hofer, M. A., & Shair, H. N. (1993). Ultrasonic vocalization, laryngeal braking, and thermogenesis in rat pups: A reappraisal. Behavioral Neuroscience, 107, 354–362.

Hofer, M. A., Shair, H. N., Masmela, J., & Brunelli, S. A. (2001). Developmental effects of selective breeding for an infantile trait: The rat pup ultrasonic isolation call. Developmental Psychobiology, 39, 231–246.

Insel, T. (1997). A neurobiological basis of social attachment. American Journal of Psychiatry, 154, 726–735.

Kalin, N. H., Shelton, S. R., & Barksdale, C. M. (1988). Opiate modulation of separation-induced distress in non-human primates. Brain Research, 440, 285–292.

Knutson, B., Burgdorf, J., & Panksepp, J. (1998). Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. Journal of Comparative Psychology, 112, 65–73.

Knutson, B., Burgdorf, J., & Panksepp, J. (1999). High-frequency ultrasonic vocalizations index conditioned pharmacological rewards in rats. Physiological Behavior, 66, 639–643.

Knutson, B., Burgdorf, J., & Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. Psychological Bulletin, 128, 961–977.

McGinnis, M. Y., & Vakulenko, M. (2003). Characterization of 50-kHz ultrasonic vocalizations in male and female rats. Physiology & Behavior, 80, 81–88.

McIntosh, T. K., & Barfield, R. J. (1980). The temporal patterning of 40–60 kHz ultrasonic vocalizations compulation in the rat (*Rattus norvegicus*). Behavior and Neural Biology, 29, 349–358.

Miczek, K. A., Tornatzky, W., & Vivian, J. (1991). Ethology and neuropharmacology: Rodent ultrasounds. In: B. Olivier, & J. Mos (Eds.), Animal models in psychopharmacology (pp. 409–427). Boston: Birkhäuser.

Nelson, E., & Panksepp, J. (1996). Oxytocin mediate acquisition of maternally associated odor preferences in preweanling rat pups. Behavioral Neuroscience, 110, 583–592.

Nelson, E., & Panksepp, J. (1998). Brain substrates of infant–mother attachment: Contributions of opioids, oxytocin, and norepinephrine. Neuroscience Biobehavioral Review, 22, 437–452.

Newman, J. D. (1988). The physiological control of mammalian vocalizations. New York: Plenum.

Newman, J. D. (1991). Vocal manifestations of anxiety and their pharmacological control. In: S. E. File (Ed.), Psychopharmacology of anxiolytics and antidepressants (pp 251–260). New York: Pergamon Press.

Panksepp, J. (1988). Posterior pituitary hormones and separation distress in chicks. Neuroscience Abstracts, 14, 287.

Panksepp, J. (1992). Oxytocin effects on emotional processes: Separation distress, social bonding, and relationships to psychiatric disorders. Annals of the New York Academy of Sciences, 652, 243–252.

Panksepp, J. (1998). Affective neuroscience: The foundations of human and animal emotions. New York: Oxford University Press.

Panksepp, J. (2003). Can anthropomorphic analyses of ''separation cries'' in other animals inform us about the emotional nature of social loss in humans? Psychological Reviews, 110, 376–388.

Panksepp, J., & Burgdorf, J. (1999). Laughing rats? Playful tickling arouses high frequency ultrasonic chirping in young rodents. In: S. Hameroff, D. Chalmers, & A. Kadzniak (Eds.), Toward a science of consciousness III (pp 231–244). Cambridge, MA: MIT Press.

Panksepp, J., & Burgdorf, J. (2000). 50 kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: Effects of social housing and genetic variables. Behavioral Brain Research, 115, 25–38.

Panksepp, J., & Burgdorf, J. (2003). ''Laughing'' rats and the evolutionary antecedents of human joy? Physiology & Behavior, 73, 533–547.

Panksepp, J., Gordon, N., & Burgdorf, J. (2002). Empathy and the action-perception resonances of socio-emotional systems of the brain. Behavior Brain Science, 25, 43.

Panksepp, J., Herman, B. H., Vilberg, T., Bishop, P., & DeEskinazi, F. G. (1980). Endogenous opioids and social behavior. Neuroscience and Biobehavioral Reviews, 4, 473–487.

Panksepp, J., Knutson, B., & Burgdorf, J. (2002). The role of brain emotional systems in addictions: A neuro-evolutionary perspective and new self-report animal model. Addiction, 97, 459–469.

Panksepp, J., Normansell, L. A., Herman, B., Bishop, P., & Crepeau, L. (1988). Neural and neurochemical control of the separation distress call. In: J. D. Newman (Ed.), The physiological control of mammalian vocalizations (pp 263–300). New York: Plenum.

Panksepp, J., Yates, G., Ikemoto, S., & Nelson, E. (1991). Simple ethological models of depression; social-isolation induced ''despair'' in chicks and mice. In: B. Olivier, J. Mos, & J. L. Slangen (Eds.), Animal models in psychopharmacology (pp 161–181). Basel: Birkhauser-Verlag.

Sales, G. D., & Pye, D. (1974). Ultrasonic communication by animals. London: Chapman & Hall.

Shair, H. N., & Jasper, A. (2003). Decreased venous return is neither sufficient nor necessary to elicit ultrasonic vocalizations of infant rat pups. Behavioral Neuroscience, 117, 840–853.

Shields, L. E. S., & Brudzynski, S. M. (2007). Ontogenetic development of acoustic features of isolation calls in Wistar rats. Canadian Journal of Neurological Sciences, 34(Suppl. 3), S11.

Thomas, D. A., Takahashi, L. K., & Barfield, R. J. (1983). Analysis of ultrasonic vocalizations emitted by intruders during aggressive encounters among rats (*Rattus norvegicus*). Journal of Comparative Psychology, 97, 201–206.

Tonoue, T., Ashida, Y., Makino, H., & Hata, H. (1986). Inhibition of shock-elicited ultrasonic vocalization by opioid peptides in the rat: A psychotropic effect. Psychoneuroendocrinology, 11, 177–184.

Tzschentke, T. M. (1998). Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects, recent progress, and new issues. Progress in Neurobiology, 56, 613–672.

van der Poel, A. M., & Miczek, K. A. (1991). Long ultrasonic calls in male rats following mating, defeat, and aversive stimulation: Frequency modulation and bout structure. Behaviour 119, 127–142.

Vivian, J. A., Barros, H. M., Manitiu, A., & Miczek, K. A. (1997). Ultrasonic vocalizations in rat pups: Modulation at

the gamma-aminobutyric acid. A receptor complex and the neurosteroid recognition site. Journal of Pharmacology Experiment Therapy, 282, 318–325.

Winslow, J. T., Hearn, E. F., Ferguson, J., Young, L. J., Matsuk, M. M., & Insel, T. R. (2000). Infant vocalization, adult aggression, and fear behavior of an oxytocin null mutant mouse. Hormone Behavior, 37, 145–155.

Winslow, J. T., & Insel, T. R. (1991). The infant rat separation paradigm: A novel test for novel anxiolytics. Trends in Pharmacological Science, 12, 402–404.