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Ultrasonic vocalizations, predictability and sensorimotor gating in the rat

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Abstract

Prepulse inhibition (PPI) is a measure of sensorimotor gating in diverse groups of animals including humans. Emotional states can influence PPI in humans both in typical subjects and in individuals with mental illness. Little is known about emotional regulation during PPI in rodents. We used ultrasonic vocalization recording to monitor emotional states in rats during PPI testing. We altered the predictability of the PPI trials to examine any alterations in gating and emotional regulation. We also examined PPI in animals selectively bred for high or low levels of 50 kHz USV emission. Rats emitted high levels of 22 kHz calls consistently throughout the PPI session. USVs were sensitive to prepulses during the PPI session similar to startle. USV rate was sensitive to predictability among the different levels tested and across repeated experiences. Startle and inhibition of startle were not affected by predictability in a similar manner. No significant differences for PPI or startle were found related the different levels of predictability; however, there was a reduction in USV signals and an enhancement of PPI after repeated exposure. Animals selectively bred to emit high levels of USVs emitted significantly higher levels of USVs during the PPI session and a reduced ASR compared to the low and random selective lines. Overall, the results support the idea that PPI tests in rodents induce high levels of negative affect and that manipulating emotional styles of the animals alters the negative impact of the gating session as well as the intensity of the startle response.

Keywords

Communication; emotion; inhibition; predictability; timing; ultrasonic; vigilance

Introduction

Prepulse inhibition (PPI) has been used in behavioral neuroscience to study diverse aspects of sensorimotor gating in humans and animal models [1, 2]. This measure of inhibition has been a powerful way to identify neural circuits and specific neurotransmitters involved in gating information [3, 4]. In addition, PPI is used frequently as a model paradigm to examine deficits in cognitive and emotional disorders such as schizophrenia, obsessive compulsive disorder and mood impairments [5–7]. Previous work using PPI has found the

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emotional state changes can influence PPI [8, 9]. Using the rodent model, PPI has been used to link brain and behavior in greater detail. Animal models of psychosis or anxiety have been tested using PPI. The measure has expanded our abilities to understand the neuropathology of mental illness and opened the pathway to new biomedical treatments targeting specific molecular and neurochemical systems [10, 11]. Results using this model could have a potentially greater impact with an emotional assessment completed during the task. Relative to the cognitive and perceptual domains, the role for emotional expression or regulation in the rodent model of PPI is not well known. The current work monitored ultrasonic vocalizations to gauge affective state in the animals during the PPI test as previous work has shown that emission of these USVs in rodents is related to different types of emotional behaviors [12–14].

USVs in adult rodents come in two major types. One is a 50 kHz call emitted during positive affective situations such as play [15], approach [16] and somatosensory hand-play [15]. The second main type of call is a lower frequency, longer duration signal around 22 kHz [17]. It is mainly emitted during negative situations such as isolation [18] or aggression [19–21] or drug withdrawal [22]. Using selective breeding, one can produce a ‘high-line’ and a ‘low-line’ ultrasound emission groups and research has shown that these two groups are significantly different on a number of emotional responses as well as the ultrasound production; this supports the idea that USV emission rates are similar to traits that can be adjusted by trans-generational selection [13, 14, 23]. One aim of the present paper was to examine whether or not animals would emit USVs during PPI testing and would the USV call type vary depending upon the type of trial the animal is exposed too. It is known that USVs are sensitive to the timing or delay of outcomes. Animals including humans show a delay discounting for outcomes that is related to anticipatory affective state (Cardinal, 2006). Knutson and colleagues (2002) found that 50 kHz USVs in rats were a part of the anticipatory period prior to reception of a drug reward [24]. Other work has found this effect using other reward types and with both 50 and 22 kHz USVs [25]. USVs in rodents could be a part of the predictive process in rats that enables them to prepare for expected outcomes or situations [16]. We examined this issue related to PPI by altering the predictive nature of the PPI test. We had three levels of predictability each one different in the timing or pattern of the PPI trial types. The three different levels include: 1) least predictive with the trial types in semi-random order and variable interval presentation, 2) intermediate predictive with the same trial order but with a fixed interval between trials and 3) most predictive with the trial types in blocks and a fixed interval. If predictability and emotional state express the typical inverse relationship found in aversive situations [26–29], then we expect that USV calls will be highest during the least predictive and lowest during the most predictive sessions.

Another aim of this study was to examine how animals bred for different levels of ultrasonic vocalizations would respond during the PPI session. A number of studies have found that this selective breeding alters a host of diverse emotional behaviors [13, 23, 30]. Recent work has shown that the high line USV group had an enhanced reaction to an aversive situation (e.g., exposure to cat odor) with prolonged suppression of play behavior [14]. A similar reaction to the PPI test would be higher 22 kHz calls by the high-line group during exposure to the loud tones. Combining work on selective breeding for USVs and gating extends the study of animals bred based on a possible emotional phenotype [31]. In general, this work on relating USVs and PPI can reveal basic interrelationships involved in gating information and emotional regulation. This type of study can be an important step toward understanding how one can interpret the findings of the PPI paradigm with animal models of mental illness more thoroughly. In particular the study could highlight how the gating process measured in this manner might incorporate or utilize emotional state information, and using USVs as a tool for uncovering the processes involved in sensorimotor gating similar to the way it is used to examine neurochemistry of normal and abnormal emotional state functions [32, 33].

The interpretations by recent work of Tunstall and colleagues (2009) relating PPI changes and drug exposure were expanded and clarified because they included ultrasound monitoring and analysis [32].

Methods

2.1 Animals

A total of ninety adult male Sprague-Dawley rats were used in the control experiments. These animals were not selectively bred. In experiment one, 60 rats were used (VI = 20, FI = 20, FI-B = 20). In experiment two, an additional 30 rats were used for the repeated exposure manipulation (VI = 10, FI = 10; FI-B = 10). The colony room is kept between 21–23° Celsius with humidity between 40–50%. All subjects were housed on a 12:12 light/dark cycle (8:00 AM and 8:00 PM on/off cycle).

The selective breeding PPI-USV experiment utilized Long Evans rats were bred at Bowling Green State University. Rats were selectively bred based upon 50 kHz USV emission during a tickle paradigm (detailed selective breeding methods are described in Webber and colleagues (2012) [14]). Breeders were at least 70 days of age. One male was bred with two females for each of the three lines of animals: high, random and low. The random line animals serve as a control for the other two selectively bred lines. Harmon and colleagues (2008) reported that typical Long Evans rats provided statistically indistinguishable responding when compared to the selectively bred random lines [13]. The colony room was the same as described previously. Selectively bred animals were pair housed for this experiment. Litter averages for males and females were used to control for litter effects (Random line: n = 9 litter; low line: n = 9 litters; high line: n = 9 litters). Animals were tested between post natal day 50 and 80 on the VI session type.

2.2 Different session types: between group exposure

The animals were tested in a PPI session which varied in terms of predictability of trial type and timing between trials. Each of the sessions was comprised of three different trials types. One type was the prepulse alone (PP, 70 dB, 30 ms) and another was the loud pulse alone (PA, 118 dB, 30 ms), and a third was the combination of the two tones (PPI, PP + PA, ISI = 50 ms). For all PPI testing, the animals were exposed to a five minute acclimation period of white noise (60 dB) that continued throughout the PPI testing session. After removal from the colony room animals were placed into the acoustic startle chamber (San Diego Instruments, San Diego, CA). USV emission was recorded throughout each session with an ultrasound BAT detector (Pettersson Elektronik, Uppsala Sweden). In the least predictable/variable interval (VI) session, animals were exposed to 60 trials of tones in a pseudorandom order at variable intervals (average ITI = 15.5 seconds). The session was programmed so that each type of trial was presented no more than two times in a row. During the predictable-fixed interval (FI) sessions animals were exposed to the identical order of trials as the VI condition, however; the intertrial interval was fixed at 15 seconds. Finally, the fixed interval- blocked (FI-B) session was comprised of the same three trial types as the other sessions; however, the trial types were presented in blocks of 20. The intertrial interval was constant at 15 seconds. The order that these blocks of trials were presented was counterbalanced. This counterbalancing procedure resulted in 6 distinct sets of blocked trials: 1) PP, PA, PPI, 2) PP, PPI, PA, 3) PA, PPI, PP 4) PA, PP, PPI 5) PPI, PA, PP 6) PPI, PP, PA.

2.3 Different session types: repeated exposure

Another way to manipulate predictability is to examine how sensorimotor gating and USV emission could be altered by repeated exposure to the PPI paradigm. We used a seven day

interval between exposures in order to reduce effects of habituation yet retain the abilities for the animals to utilize the previous exposure in terms of contextual memory effects. For experiment two, a separate group of animals was exposed to the VI, FI or FI-B session twice. There was a seven day break between the two exposures. USVs were recorded for both week 1 and week 2 exposures.

2.4 Data Analysis

All of the statistics were calculated using SPSS (Version 15.0, SPSS, Inc., Chicago, IL). The alpha level utilized was 0.05. Non-parametric tests were used in the analysis because data violated assumptions of normal distribution. Kruskal-Wallis and Friedman's tests were used for omnibus tests. Mann-Whitney and Wilcoxon signed rank tests were used for independent and paired comparisons, respectively. Pearson correlation statistics were used to investigate relationships between ASR, PPI and USV emission. ASR was averaged over trial type measured in units of 1.22 mV. These trial averages were calculated into a ratio to assess PPI: $100 - [(prepulse + pulse / pulse\ alone) \times 100]$ (Swerdlow et al., 1990). Note that the PP trials are not used in calculating PPI(%), these trials were added to examine how predictable and unpredictable less aversive, quiet pulses may interact with fear, USVs and predictability. USV data was scored manually on a computerized spectrogram (Avisoft Pro, Germany). USV emission was taken as a function of seconds (number of USVs/sec) in order to compare results among the different PPI sessions. For experiment 2 averages of ASR and USV emission during entire sessions were averaged and used to examine differences over the 2 rounds of testing.

Results

Ultrasound emission during PPI testing

Ultrasound production results were very clear and high levels of 22 kHz USVs were produced throughout the sessions. Figure 1 provides a histogram of the 22 kHz USV calls in the different trial types and accentuates the differences among the sessions. The FI-B example is one with the order low tone (PP) first followed by the two loud tone trial types (PA and PPI). High rates of 22 kHz USVs were emitted during PPI testing, ranging from 0.1 to 1.5/sec. It is clear that in each trial type animals produce a steady level of USVs throughout the session except for the FI-B session which begins with the lower level tone (20 trials in blocked presentation of the PP soft tone trials; Figure 1). There was a main effect of session type on USV emission during the PP trials (see Figure 2; $H(2) = 7.638$, $p = .022$). Animals in the FI-B session produced significantly fewer 22 kHz USVs during the PP trials when compared to the VI ($U = 121$, $p = .033$) and the FI session ($U = 106$, $p = .010$). This result could indicate a period of relief from aversive trial types during the most predictable session (FI-B). We found significant relationship between startle and USV emission in the FI-B session. ASR during the loud tone trials (PA trial set) had a significant negative correlation with PA USV emission ($r(18) = -.485$, $p < .05$). This result suggests that USV declines in the most aversive trials as startle amplitude increases but only in the most predictable situation. One possible mechanism for this might be the habituation to the loud tone aversiveness that occurs without a concomitant shift in startle amplitude.

An interesting way to examine USV emission during PPI is to incorporate the relative levels of USV calls in the loud tone trials with and without a prepulse. We examined USVs in a fashion appropriate for the PPI paradigm and utilizing the standard PPI equation. We replaced the ASR values with USV numbers in this PPI equation ($100 - (PPI_USVs / PA_USVs) \times 100$) in order to determine whether or not an inhibition of USVs could be occurring throughout the session. There was a main effect of session type on USV inhibition (see Figure 3; $H(2) = 13.489$, $p = .001$). Results showed that USV inhibition varied

significantly between the session types (Figure 3) with the predictable session (FI) having the most effective inhibition compared to the variable interval session ($U = 86$, $p = 0.002$) and the FI-B session ($U = 82$, $p = 0.001$). Surprisingly, in the FI-B session the USVs are facilitated in the PPI trials compared to the PA trials, the complete opposite effect observed in the other sessions and contrary to the effect observed for the inhibition of the ASR. In order to understand the effect better, we divided the FI-B sessions into the 6 different orders and measured the USV inhibition score for each one (Figure 4). It was found that if the PA trials come before the PPI trials, there is a USV facilitation effect ($U = 95$, $p = 0.007$). The only exception was when the PP trials came after the PA trials, and before the PPI trials. These PP trials in between the PA and PPI trials probably acted as an “emotional buffer”. The next series of analyses examined the dependent measures within the different session types.

PPI and ASR in different sessions

There was a main effect of session type on PP ASR ($H(2) = 8.695$, $p = .013$). Exposure to the most predictable session type (FI-B sessions) produced an increased ASR when compared to the VI session PP trials (Figure 5; $U = 110.5$, $p = .014$) and FI session PP trials (Figure 5; $U = 108$, $p = .007$). When comparing the PPI scores among the three sessions, no significant differences were obtained (Figure 6). Even though the startle response did vary among session types, it was only for the PP trials which were not included in the PPI equation. Results showed that the least aversive PP trials were relatively less aversive during the more unpredictable session types.

Comparing differences depending upon trial types for each session

VI Sessions: There was a main effect of trial type on USV emission in the VI session ($X^2(2) = 8.333$, $p = .000$). Animals produced significantly more USVs in response to the PA when compared to the PP ($Z = -2.867$, $p = .004$; Figure 2A) and PPI trials ($Z = -2.108$, $p = .035$; Figure 2A). During the VI session animals produced significant differences in ASRs ($X^2(2) = 23.700$, $p = .000$; Figure 5A). Animals produced a greater ASR during the PA trials when compared to the PP and PPI trials ($Z = -3.92$, $p = .000$; $Z = -3.92$, $p = .000$). Animals also produced a greater ASR during the PPI trials when compared to the PP trials ($Z = -3.783$, $p = .000$). *FI Session:* There was a main effect of trial type on USV emission in the FI session ($X^2(2) = 16.734$, $p = .000$). Animals produced significantly more USVs in response to the PA when compared to the PP ($Z = -2.938$, $p = .003$; Figure 2B) the PPI trials ($Z = -3.397$, $p = .001$; Figure 2B). During the FI session animals produced significant differences in ASRs ($X^2(2) = 38.100$, $p = .000$). Animals produced a greater ASR during the PA trials when compared to the PP ($Z = -3.92$, $p = .000$; figure 5B) and PPI trials ($Z = -3.833$, $p = .000$; figure 5B). Animals also produced a greater ASR during the PPI trials when compared to the PP trials ($Z = -3.928$, $p = .000$; figure 5B). *FI-B Session:* There was a main effect of trial type on USV emission in the FI-B session ($X^2(2) = 19.645$, $p = .000$; figure 2C). Animals produced significantly more USVs during the block of PA and PPI trials when compared to the PP trials ($Z = -2.745$, $p = .006$; $Z = -3.637$, $p = .000$; Figure 2C). During the FI-B session, there were significant differences in ASRs between the different sequences of blocked trial sets ($X^2(2) = 27.700$, $p = .000$; Figure 5C). Overall, animals produced a greater ASR during the PA trials when compared to the PP ($Z = -3.92$, $p = .000$; Figure 5C) and PPI trials ($Z = -3.883$, $p = .000$; Figure 5C). Animals in this session did not produce a greater ASR during the PPI trials when compared to the PP trials. In order to understand the effects within the FI-B trials more thoroughly, we divided the FI-B sessions into the six different orders and examined differences among this group. Since there was not a main effect of order on ASR ($p > .05$), PPI(%) ($p > .05$) or USV emission ($p > .05$) we did not pursue this analysis any further.

Effects of repeated PPI session exposure

VI Results: There was a significant difference in average USV emission over the 1st and 2nd session of testing. Animals produced significantly fewer USVs overall during the second round of testing when compared to the first round of VI testing ($Z = -1.988$, $p = .047$). Specifically, they produced significantly more 22 kHz USVs during the PP trials in round 1 than in round 2 ($Z = -2.599$, $p = .009$; Figure 7A). There were no significant differences in PPI (%) or ASR between the two rounds of testing in this session ($p > .05$). **FI Results:** There was a significant difference in average USV emission over the 1st and 2nd session of testing. Animals emitted fewer 22 kHz USVs in the second round of testing when compared with the first round of testing ($Z = -2.521$, $p = .012$; Figure 7B). Animals in the FI session emitted more USVs during the PA trials in the first round of testing when compared to the second round ($Z = -2.527$, $p = .012$). Interestingly, the within-session differences among the trials types for startle amplitudes was abolished in the second week of testing, that is to say the startle levels were similar in week 2 among the PP, PA and PPI trial types. Animals in the FI condition showed significantly greater PPI(%) in the second round of testing when compared to the first round ($Z = -2.803$, $p = .005$; Figure 8B). There were no significant differences in ASR between the two rounds of testing. **FIB Results:** There was not a significant difference in average overall USV emission between the 1st and 2nd session; Figure 7C). However, animals produced significantly more 22 kHz USVs selectively during the PP trials in round 2 when compared to round 1 ($Z = -2.023$, $p = .043$; Figure 7C). No significant differences in PPI (%) or ASR between the two rounds of testing in this session were found.

Selective Breeding and USV emission during PPI testing

There was a main effect of animal line on 22 kHz USV emission during all three trial types (PP: $H(2) = 12.265$, $p = .002$; PA: $H(2) = 12.701$, $p = .002$; PPI: $H(2) = 13.607$, $p = .001$). High line animals produced significantly more 22 kHz USVs than the random line animals during all three trials types (PP: $U = 13.500$, $p = .014$; PA: $U = 13.500$, $p = .014$; PPI: $U = 12.500$, $p = .011$; figure 9) and significantly more than low line animals during all three trial types (PP: $U = 7.000$, $p = .002$; PA: $U = 6.000$, $p = .002$; PPI: $U = 5.000$, $p = .001$; figure 9). High line animals significantly varied their USV emission over trial type in the VI session ($X^2(2) = 14.250$, $p = .001$; figure 9C). Specifically they showed significantly more 22 kHz USV emission during the PA ($Z = -2.521$, $p = .012$) and PPI trials ($Z = -2.521$, $p = .012$). High line animals also showed significantly more 22 kHz USVs in response to PPI trials than the PP trials in the VI session ($Z = -2.380$, $p = .017$). Random line animals marginally varied their USV emission over trial type in the VI session ($X^2(2) = 6$, $p = .05$; figure 9A), showing a non-significant increase in USV emission during the PA and PPI trials when compared to the PP trials ($p > .05$).

Selective Breeding and ASR/PPI(%) during PPI testing

There was a main effect of animal line on ASR during the PA trials ($H(2) = 8.914$, $p = .012$), but not the PP or PPI trials ($p > .05$). Random line animals produced a significantly greater ASR than the high ($U = 15.500$, $p = .024$) and low line ($U = 9.000$, $p = .004$) animals during the PA trial types (figure 10). There was a main effect of trial type on ASR in the VI session ($X^2(2) = 31.185$, $p = .000$). Random line animals produced a significantly greater ASR in response to PA trial types when compared to PP ($Z = -2.666$, $p = .008$; figure 10A) and PPI trial types ($Z = -2.023$, $p = .043$; figure 10A). PP and PPI trials marginally differed within the random line ($Z = -1.955$, $p = .051$). Low line animals produced a significantly greater ASR in response to PA trial types when compared to PP ($Z = -2.666$, $p = .008$; figure 10B) and PPI trial types ($Z = -2.666$, $p = .008$; figure 10B). Low line animals also showed greater ASR during the PPI when compared to the PP trial types ($Z = -2.073$, $p = .038$; figure 10B). High line animals produced a significantly greater ASR in response to PA trial types when

compared to PP ($Z = -2.666$, $p = .008$; figure 10C) and PPI trial types ($Z = -2.666$, $p = .008$; figure 10C). High line animals showed a significant relationship between ASR and 22 kHz USV emission during the PA trials in the VI session ($r = .668$, $p = .049$). There were no other significant correlations between USV emission and ASR. There was not a main effect of animal line on PPI(%) score ($p > .05$).

Discussion

A main finding of the study was that 22 kHz ultrasonic signals are emitted at consistent and relatively high rates throughout the test of sensorimotor gating. The histogram of USV calls during the PPI session (Figure 1) suggests that there is a warm up period in which the calls gradually rise and then are observed throughout the session. Predictability did significantly influence the USV emission with higher levels of predictability lowering USV call levels. The USV inhibition score calculated from the different sessions varying by degree of predictability revealed that USV calls were inhibited during exposure to predictable fixed interval session; however, this effect was reversed when the trial types were presented in blocks of identical trials and in particular when the initial block of trials was comprised of consecutive loud tone presentations. This shows that USVs like the ASR can be inhibited during the PPI paradigm, but is altered even more by the order of aversive tones when presented in long blocks of trials. Generally, 22 kHz USVs reduced over repeated testing, suggesting that prior exposure to the paradigm reduced some of the aversive aspects of testing. This did not occur during the ASR measure, suggesting that these behaviors reflect different aspects of emotional processing.

The results suggest dissociation between measures obtained during testing. ASR may be more suited for measuring short affect occurring over variable time courses, while USV emission may be more suited for large affect occurring over more stable conditions. ASR may better represent fear while 22 kHz USVs may better represent anxiety [34–37]. The relative reduction in ASR during the soft tone trials in the unpredictable versus predictable session types may reflect a relative reduction in fear during those short and very specific time points without showing a general reduction in anxiety. Conversely, the reduction in 22 kHz USV emission during the PP trials over an extended block may reflect reduced anxiety during the soft tone block of trials, but not fear in response to the tones in general [38]. USV emission only rose and fell with ASR during the most aversive trial types, in the most predictable blocked session type. This suggests that they may only reflect the same aspects of negative affect when both aversion and predictability are at their highest. This work highlights the value of using different emotional markers to gain a richer understanding of affective processes.

PPI did not vary among session type during initial testing. The lack of any effect of predictability on the initial PPI measure in these experiments suggests that sensorimotor gating is resilient to changes in predictability during initial testing. However, PPI did vary during the repeated measure testing but only during the predictable FI session type. This effect did not occur during the unpredictable VI or the FI-B session types, suggesting that the prior exposure to VI testing didn't enhance sensorimotor gating during future exposure. This lack of effect in both the VI and FI-B session types is probably attributed to different reasons. The VI session types were the most aversive and least predictable. The complete inability to expect future trial types in the VI session most likely prevented any enhancement in sensorimotor gating to be gained from prior exposure. Conversely, the extremely high predictability of the FI-B session type led to very consistent emotional and gating responses that were inflexible regardless of prior exposure. The FI session type seemed to provide a "goldilocks zone" which resided between very low and very high predictability in which expectancy enhanced by prior experience could modulate reflexive sensorimotor gating

processes. This “goldilocks zone” seemed to provide the ideal level of predictability that allowed for expectancy effects to emerge, but only during repeated testing [39, 40]. Taken together, this work shows that while it is very difficult to gain changes in sensorimotor gating that coincide with changes in expectancy, it is possible under scenarios of moderate predictability and only after prior experience.

USV emission was found to be higher in the high-line animals during the PPI test and this is consistent with recent findings that these high-line USV emission animals are more reactive to stressful events [14]. A byproduct of selecting for high levels of 50 kHz USVs may have been to select for emotional reactivity or elevated emotional intensity in both the positive and negative directions. Other work has shown that these animals do emit fewer negative USVs under social stress or during development but these tests (e.g., social isolation) are not as aversive to the animal in terms of the duration or intensity of the PPI test. The use of selectively bred animals to examine gating [41] is another way to reveal trait characteristics related to emotional modulation or disruption can alter a sensorimotor or perceptual process which can influence human gating mechanisms [42–44] or gating in animal models of fear or anxiety [45]. Emotional states induced by stress [46, 47] or social isolation [37, 48] can significantly disrupt PPI. Stress in rats can disrupt or enhance gating depending upon the duration and intensity of the stressor and the neural or behavioral measure being used to evaluate gating [47, 49, 50].

We found a high rate of 22 kHz call emission (0.6–1.2 calls/s) that persisted across the PPI session. Every animal tested emitted the calls in a consistent fashion. At the highest level (>1.0 call/s) animals are emitting over 1200 calls in a twenty minute session. Listening to this rapid, continuous calling is striking and does indeed portray a negative emotional experience for the animal during the typical session. 22 kHz USVs have been recorded in numerous negative affective paradigms including fear conditioning [51], aggressive social experiences including social defeat and predator exposure [18, 22, 52], drug withdrawal (Covington and Miczek, 2003), chronic pain [53, 54] and after experience to startling tones [55]. Typically 22 kHz calls are not observed when high aversiveness is not present. For example, they have not been found in studies with reward devaluation or negative contrast [56]. There might be an important negative affective threshold in order for these specific calls to be emitted and general states of frustration or reward extinction may not be sufficient. The threshold for negative affect in rats would be worthwhile to explore as animal models of depression and other mental illnesses attempt to mimic negative affect and its behavioral consequences. Exploring parameters of USV emission would help advance this goal. The rate of 22 kHz USVs does vary in different paradigms using aversive stimuli but mainly ranges between 0.3 to 0.6/s. Even in highly aversive tone-shock conditioning. During the actual conditioning period there is significant variability in the probability and level of emission of 22 kHz calls [57]. Monitoring USVs in this previous work aided the researchers in their ability to understand variability not only in the USV measure but also in other measures of emotional behavior (Borta et al., 2006; Schwarting et al., 2007). Several factors were found to be important in moderating USV emission levels and external factors such as housing type and other experiences interacted with internal factors such as rat strain and inherent traits related to emotional predispositions [58]. Our previous work using USV levels to produce high-line and low-line USV emission (50 kHz calls) also found a link among USV emission within the selectively bred lines and emotional behaviors [13, 14, 23]. This work using selective breeding of USV levels demonstrates the power of USVs as a phenotypic marker for emotional responses in diverse situations throughout development.

The higher average rate of USV emission observed in the present study may be related to the longer duration session and the more persistent exposure to an aversive stimulus. Given this high rate of USV emission, it was somewhat surprising that PPI levels did not co-vary with

USVs in the different sessions. Previous work using fear conditioning did observe a significant positive correlation between USV emission and freezing behavior [57]. These paradigms are both aversive to the animal there are potential important ways that the behavioral measures of freezing and startle differ making direct comparisons difficult. One way they differ is how the behavior temporally relates to the real-time USV emission. During the PPI session, the constant USV emission is completed and measured prior to the loud tone exposure and is dependent upon the predictability of the tone onset. During fear conditioning, the USVs measured are typically during the conditioned stimulus duration which is also highly predictive of the unconditioned stimulus-shock. In our paradigm there is no discrete CS except for timing and USVs may be emitted as a way to track temporal dynamics throughout the session. Another important difference between these aversive situations is the behavior measured. Freezing is highly dependent upon forebrain modulation while PPI and ASR can be dissociated from forebrain regions and has been shown to be highly dependent upon brainstem mediation in certain contexts [4, 59, 60]. It could be the type of active nature of startle reflex or the greater reliance upon bottom-up processing that enables mechanisms related to startle and PPI be disconnected from emotional state changes [61].

USVs and PPI have been examined together in a previous study [32]. They found that USV emission was important in revealing alterations in drug-induced memory deficits that influenced gating over time. The study administered phencyclidine to animals over a period of days and examined changes in PPI as the animals experienced multiple sessions. Similar to our study, they found multiple experiences with PPI can change the level of inhibition and the level of USVs emitted [32]. The overall levels of emission were lower than the present study (0.3 to 0.5 calls/s) but a key difference includes the data presented arising after the animals had experience with the chamber and the restraint device. This initial exposure could lead to a downshift in negative affect and stress responsiveness. Monitoring USVs was especially helpful for this particular pharmaceutical study on gating because it enabled the researchers to pinpoint dynamic changes in emotion that can occur over time and how these reflect a learning or habituation process that recruits working memory. The exposure to the drug altered this cognitive process and led to a reduction in the multiple exposure effect. In general our findings point to a need to revise how we measure and develop our animal models of mental illness in order to gain information that helps in translating the information to clinical settings [62]. Gating of information is a pervasive neural mechanism with distinct functions depending upon neural network processes [49, 50, 63, 64]. This work provides an example illustrating the power of obtaining measures of diverse psychological functions in parallel and attempting to uncover relationships among them to elucidate interactions and diverse ways information processing can be impaired.

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Highlights

- Sensorimotor gating is influenced by emotional state
- Prepulse inhibition is an established model of gating
- We examined ultrasound production by control and selectively bred (USV line) rats during PPI
- Ultrasounds are produced during PPI testing
- Ultrasound production depends on predictability and genetic bred line status

Figure 1.
Histograms of USV emission for the 3 different sessions from experiment 1.

Figure 2.

Ultrasonic vocalizations among sessions. Animals in both the VI and FI condition showed elevated levels of 22 kHz USV emission during the PA trials when compared to the PP and PPI trials. Animals in the FI-B session show elevated levels of USV emission in response to the PA and PPI trials when compared to the PP trials. * $p < .05$

Figure 3.

A calculation of USV inhibition was determined by inserting the USV numbers per trial types into the standard PPI equation. We obtained significant differences amount the session types with the FI trial significantly more inhibited compared to the VI session. The FI-B session was actually facilitated in USV calls meaning animals were emitting more in the loud tone trials without the prepulse compared to the trials with the prepulse. * $p < .05$

Figure 4.

We performed an analysis using the USV inhibition score among the different session types in the FI-B series. The facilitation effect was mainly due to the presentation of 20 trials of loud tones in a row prior to the prepulse trials leading to a ‘sensitization’ of startle and abolishing of the prepulse inhibitory effect.

Figure 5.

Acoustic startle response measure among the sessions. Animals in both the VI and FI condition showed enhanced ASRs during the PA and PPI trials. They also produced greater ASRs during the PPI trials when compared to the PP trials. Animals in the FI-B session show only a significantly elevated ASR during the PA trials when compared to the PP trials.

* $p < .05$

Figure 6.

Prepulse inhibition scores among the sessions. PPI scores did not significantly vary among the different sessions that varied on levels of predictability.

Figure 7.

Ultrasound emission during each session and trial type over rounds of testing. Overall animals showed general reductions in USV emission over rounds of testing. During the VI session animals significantly reduced USVs during PP trials, during the FI session animals significantly reduced USVs during PA trials, and during the FI-B session there was a significant increase in 22 kHz USV emission during the PP trials in the second round of testing. * $p < .05$

Figure 8. Prepulse inhibition in the fixed interval blocked sessions. Animals in the FI condition alone showed an enhancement of PPI(%) during the second round of testing when compared to the first. * $p < .05$

Figure 9.

Ultrasound emission in the animals selectively bred to emit high versus low levels of 50 kHz calls during the standard PPI Session (VI session). High line animals emit significantly more USVs during PA and PPI trials when compared to PP trials. * $p < .05$

Figure 10.

Acoustic startle response in selectively bred line animals. High and low line animals showed a significant reduction in ASR compared to random line animals and this reduction depended upon the trial type (PA trials only) experience during the session. Random and low line animals produced a greater ASR during PA and PPI trials when compared to PP trials. High line animals showed greater ASR during PA trials than PP and PPI trial types. * $p < .05$