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Research report

Selective breeding for 50 kHz ultrasonic vocalization emission produces alterations in the ontogeny and regulation of rough-and-tumble play

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ABSTRACT

Ultrasonic vocalizations (USVs) are emitted by rodents and can signal either negative or positive affective states in social and nonsocial contexts. Our recent work has utilized selective breeding based upon the emission of 50 kHz USVs in response to standard cross species hand play—namely experimenters 'tickling' rats. Previous work has shown that high-tickle responsive animals (i.e., rats emitting abundant 50 kHz USVs) are gregarious and express enhanced positive emotional behaviors relative to animals exhibiting low 50 kHz USVs. The present study extends this work by examining the developmental profile of play behavior and the suppression of play behavior by predator (cat) odor in juvenile high-line and low-line animals. Results support dissociations in key play measures between these groups, with high-line animals emitting more dorsal contacts during play and low-line animals emitting more pinning behavior. For cat-odor induced play suppression, we found that high-line animals exhibit elevated suppression of play for a prolonged period compared to low-line rats. In contrast, low-line animals returned to normal levels of play just 1 day post-predator odor experience. These findings support the idea that emotional arousal may differ between these selectively bred groups, and extends previous work by demonstrating a possible influence of altered emotional learning and conditioning in these phenotypically different animals. One possibility is that high-line animals exhibit enhanced associative learning abilities leading to stronger negative contextual conditioning. These findings suggest that selection for positive or negative social–emotional phenotypes may also segregate genes that control emotional learning abilities in unanticipated ways.

1. Introduction

It is clear that the majority of animal models commonly used to study emotional impairments of mental illness do not adequately 'model' related altered affective states. This is because affective states are rarely the target of animal model development. Emotional impairments are difficult to model compared to other processes because there are currently few validated measures of affect or its impairment in common model organisms such as laboratory rodents. Recent work monitoring ultrasonic vocalizations (USVs) is providing a new window to the neurology of underlying affective processes that may more directly model constitutional emotional changes compared to previous efforts [1–6]. Rodent USVs range from 22 kHz to 70 kHz and can reflect different affective states [3,7–10]. For example, 50 kHz USVs are emitted by rats in response to and in anticipation of pharmacological as well as conventional rewards, especially positive social stimuli [3,6,11]. Conversely, 22 kHz USVs are emitted in response to and in anticipation of administration of aversive drugs and environmental events and negative social stimuli [10,12,13]. Indeed, the 50 kHz USVs arise from brain systems that are rewarding while 22 kHz USVs arise from circuits that are punishing [12–16]. Individual differences in emotional responses have been found in rats that that emit high or low amounts of 50 kHz USVs during a "tickling" paradigm [17]. Rats that emit few 50 kHz USVs during the "tickle" produce more behaviors that are reflective of negative affect after exposure to chronic stress when compared to rats that emit many 50 kHz USVs during the same paradigm [17]. Specifically, they show lower sucrose preference during sucrose preference testing, more immobility behavior in the forced swim test and more dramatic weight reduction compared to rats that emit greater levels of 50 kHz USVs during "tickle" [17]. Additionally, the low USV emitters show greater oxidative metabolism in brain areas involved in negative affect [17]. These results strongly suggest that 50 kHz USV

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emission during "tickle" can be used to examine predispositions to negative affective states and vulnerability to stress.

For the past decade, we have selectively bred rats of the Long–Evans strain for low or high levels of 50 kHz USV emission [18]. An important goal of this work has been to segregate the alleles that are related to positive and negative social affect [11,13,19]. Animals have been bred based upon the number of 50 kHz USVs emitted during a standard "tickle" assay, which aims to simulate the rough-and-tumble play of rats. As a result of such systematic cross-species social experiences, young rats learn to follow the experimenter's hand around the "tickle" chamber and express dorsal contacts or play bites as apparent solicitations for more "tickle" play. An effective targeted stimulation appears to be to the anterior dorsal surface of animals, even though full-body tickling (targeting ventral and dorsal body regions) is most effective. Five generations of selective breeding sufficed to generate constitutional differences between lines of animals in both USV emissions as well as related social behavior [18]. The resulting high line (HL) rats emit significantly more 50 kHz USVs than the low line (LL) animals, in a variety of tests including conditioned "tickle" reward paradigms as compared to 'random' bred (RL) control animals [18].

Among their natural interactions, one of the best elicitors of positive 50-kHz USVs in rats is the social behavior of rough-and-tumble play behavior [13]. Rough-and-tumble play behavior is highly rewarding in rats, and the reward value is positively correlated with rates of 50-kHz USVs [19]. To monitor their natural play urges, two especially robust and easily monitored behaviors have traditionally been employed as indicators of playfulness, dorsal contacts and pins [16]. Dorsal contacts refer to one animal touching the dorsal surface of the other with one or two paws. Pins refer to one animal transiently achieving a "top dog" posture with the presumably submissive partner momentarily on its dorsal surface [16]. In the midst of play rats also emit abundant USVs, with uniformly high 50-kHz USVs early in play episodes. It has been hypothesized that 50-kHz USVs index the rewarding effects of play and may function to facilitate play and other positive social engagements that help promote social bonds and ultimately reproduction [19].

Predator odor is a powerful and natural unconditioned fearful stimulus for rodents [16,20–22]. The introduction of cat scent into an arena will produce a robust fear response in rats [20–22]. This fear response arises from an innate process, as the rats do not need to have any experience with cats or cat scent to elicit it. Panksepp [16] has reported that play behavior in juvenile rats was severely inhibited by the presence of cat hair, and this effect persisted over several days. Siviy et al. utilized a worn cat collar instead of tufts of cat hair to enable greater stimulus control [23]. They found that the worn cat collar significantly reduced play, remaining below initial baseline levels for at least 6 days. The aim of the present work was to examine the basic developmental profile of play and the intensity of play suppression by predatory odor in animals from the different breeding lines based on different levels of 50 kHz vocalization emission. This extends previous studies by examining in detail the ontogenetic profile of these lines and by incorporating not only analysis of the unconditioned fear response but also adding in an analysis of the conditioned suppression of play during extinction. The general anticipation for the results included higher and lower levels of play in the HL and LL animals, respectively.

2. Materials and methods

2.1. Animals

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. 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, Ohio) with one male per female. RL animals were created through arbitrary pairing of HL and LL animals. They represent the control group for the selectively bred lines. In experiment 1 a total of 66 animals were used (RL: $n = 22$; HL: $n = 22$; LL: $n = 22$). In experiment 2 a total of 60 animals were used (RL: $n = 20$; HL: $n = 20$; LL: $n = 20$). For this study, only litters yielding 8 or more pups were used for testing. At 21 days of age, juvenile animals to be tested were separated from the litter and individually 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 h) 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.

2.2. Play behavior development

After being placed into individual housing (PND 21), testing was conducted the next day and every other day for the next 11 days, namely at 22, 24, 26, 28, 30 and 32 days of age. The paradigm has been one used as a standard method to facilitate play behavior in rodents [16]. The play arena, located in a separate testing room, was a box (30.5 cm × 30.5 cm × 50.5 cm) with three stainless steel sides and one Plexiglas side and floor. The clear Plexiglas side allowed for video recording. A testing session consisted of removing a pair of animals from their home cage in the colony room and placing them in the play arena for 5 min. Animals were chosen as play partners if they were within 10% of their body weight and the same sex. Animals were immediately returned to their home cage post-testing.

2.3. Play behavior suppression and extinction

At 24 days of age, same sex littermates were paired based upon similar weights, and then placed in a 30 cm × 30 cm × 30 cm Plexiglas play chamber with corn cob bedding. Bedding was changed in the play chamber between testing sessions of animals of a different sex or from a different litter. The play chambers had black paper taped to three of the sides and testing was conducted under dim white light. Two chambers were utilized in this paradigm, one for the worn cat collar and the other for the rest of the testing days. The rats were exposed to 3 days of acclimation to their play partner and experimental context. Before each play bout one of the animals in each pair was marked with a Crayola magic marker for identification purposes. The same animals were marked throughout the play testing. Each play bout lasted 5 min.

Video recording did not take place during the acclimation phase on post-natal days (PND) 23, 24, and 25. On the fourth day (PND 26) the animals were video recorded during their play bouts (DVD format: Panasonic DVD recorder and camcorder). This served as the baseline measure of play behavior for the comparison of extinction testing sessions. On the fifth day (PND 27) four 2 cm strips of unworn cat collar placed into the play chamber. The worn and unworn cat collars were stored in a freezer to keep the scent fresh. Before testing the cat collars were placed into zip top bags and warmed up in 55 °C water for 10 min. They were then placed into each corner of the play chamber and buried in the corn cob bedding, this served as a control for the worn cat collar. The collars were worn for at least 2 weeks by a 2-year-old male cat. On the sixth testing day (PND 28), four 2 cm pieces of warmed worn cat collar were placed in each corner of the worn cat collar play chamber in the same manner. Eight consecutive days of play (PND 29–PND 36) without any collars were conducted after the worn cat collar exposure. This was done to investigate the time required for the rats play behavior to return to baseline levels (i.e., extinction phase). All play behavior was videotaped to permit viewing of behavioral interactions. USVs were recorded on the worn cat collar day (Pettersen D980 Ultrasonic Detector, Uppsala, Sweden). USVs were not monitored on the other days of play due to noise resulting from vigorous play behavior and locomotor activity in the bedding. The lack of play during the worn cat collar exposure allowed an accurate count of USV emission due to the absence of background noise from the bedding.

2.4. Videotape analysis

Videotaped play behavior was scored by a trained experimenter in real-time for two major action categories: dorsal contacts and pins (OD-LOG, Macropod Software, Version 2.5.2). Behavioral scoring was conducted by an experimenter blind to all testing conditions. Play behavior was scored from both animals during each session. This comprehensive scoring could lead to mutual exclusive behaviors expressed between members of the pay dyad. This mutual exclusivity should be similar among the three groups given the identical scoring methods. A pin was scored if one animal was lying on its back with the other animal on top. A dorsal contact was scored if an animal touches the other with its front paws on the dorsal surface between the neck and the rump, not including the tail [24]. Behavioral scoring was performed with computer-assisted software and equipment designed to accurately tally the incidents of dorsal contacts, pins and pin duration (seconds). A sample of videos of each of the selectively bred lines of animals during play suppression has been added as supplementary material to this paper in the form of .avi files.

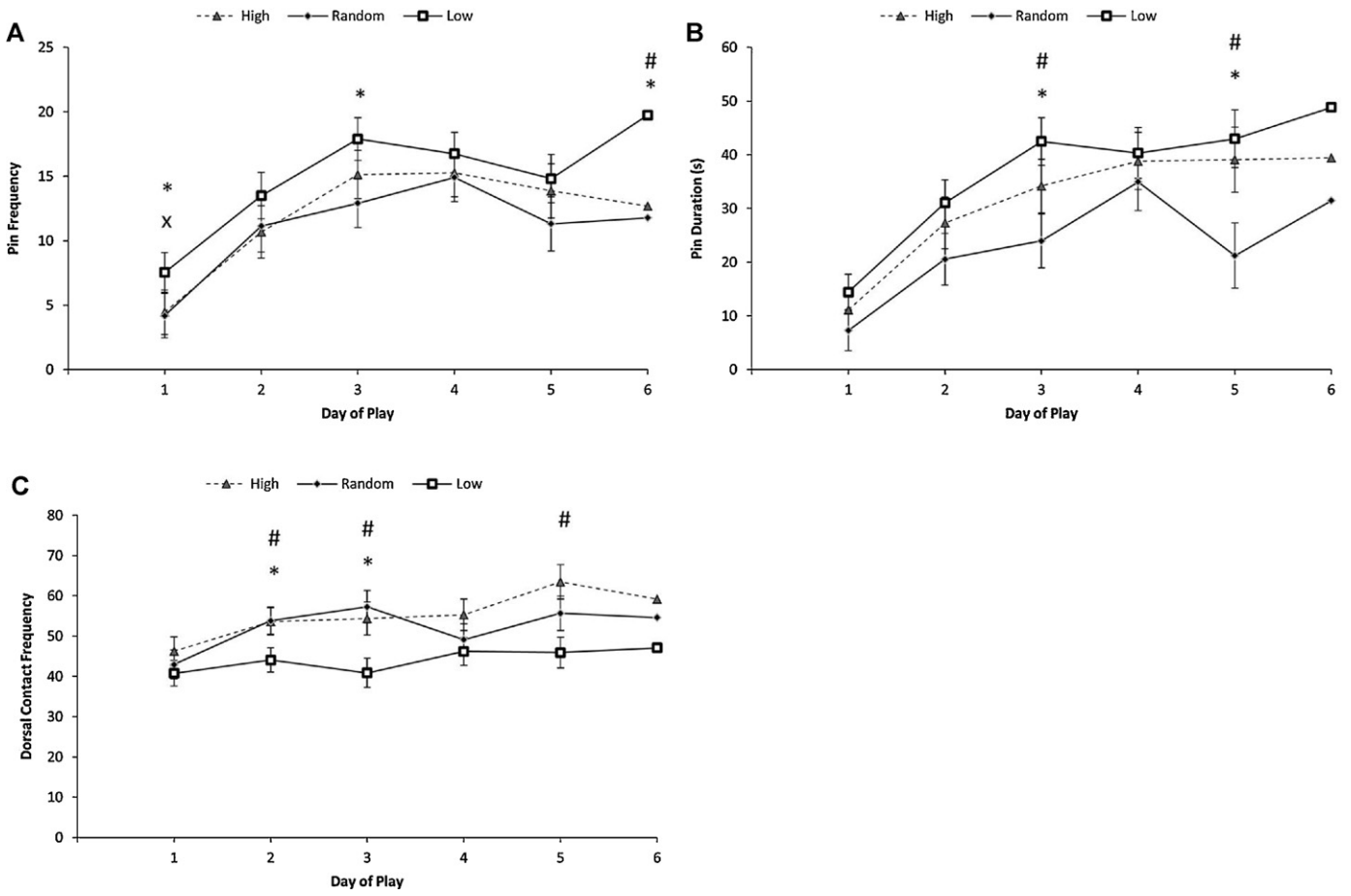

Fig. 1. Play behaviors measured during play development. Pin frequency, pin duration and dorsal contact frequency varied significantly within and among the selectively bred lines during the play development experiment. (A) All three selectively bred lines of animals produced significantly more pins on the 2nd–6th day of play when compared to the 1st day of play ($p < .01$ for all comparisons). (B) All three selectively bred lines of animals produced significantly longer pins on the 2nd–6th day of play when compared to the 1st day of play ($p < .01$ for all comparisons). (C) The high line animals produced significantly more dorsal contacts on the 5th day of play when compared to the first ($p < .01$). RL animals produced more dorsal contacts on the 3rd day of play when compared to the first ($p < .01$). LL animals did not show significant increases in dorsal contacts over the play experiment ($^{*}p < .01$, LL versus RL comparisons; $^{\times}p < .01$, HL versus RL comparisons; $^{\#}p < .01$, HL versus LL comparisons).

2.5. Data analysis

Between subjects data was analyzed with Mann–Whitney *U*- tests. Within subjects data was analyzed with Wilcoxon signed ranked tests. Non-parametric statistical tests were used because the data violated the assumption of normal distribution. For multiple within-subjects comparisons the Sidak–Bonferroni correction was used. For experiment 1 $\alpha = .02$ and for experiment 2 $\alpha = .01$.

3. Results

3.1. Play development

Each of the behavioral indicators of play increased in the RL over the initial period (first 3 days) of testing. This is similar to previous work on the ontogeny of play in rats [25]. The other lines (HL and LL) were not as consistent in the early period with both increases (i.e., pinning behavior) and decreases (i.e., dorsal contacts) observed. Analyses of differences in these different actions were completed for each testing day. LL animals pin frequency was higher than that of the RL on the 1st ($U = 147$, $p = .024$), 3rd ($U = 157.5$, $p = .04$) and 6th ($U = 137.5$, $p = .014$) days of play (Fig. 1A). LL animals produced significantly more pins than the HL later in development on the 6th day of play ($U = 154.5$, $p = .04$). Interestingly, HL animals pin frequency was significantly greater than the RL on the first day of play ($U = 159.5$, $p = .05$).

Pin duration differences were seen in the middle and later part of the testing period. LL animals produced longer pins than the RL on the 3rd ($U = 151$, $p = .033$) and 5th ($U = 136$, $p = .013$) days of play (Fig. 1B). HL animal pins were longer than the RL on the 3rd ($U = 155.5$, $p = .042$) and 5th days of play ($U = 140.5$, $p = .017$).

The largest effects were observed for dorsal contacts with significant divergence between the HL with enhanced and LL depressed levels of behavior. LL animals produced significantly fewer dorsal contacts than the RL on the 2nd ($U = 128.5$, $p = .008$) and 3rd ($U = 97$, $p = .001$) days of play (Fig. 1C). HL animals produced significantly more dorsal contacts than the LL on the 2nd ($U = 121$, $p = .004$), 3rd ($U = 133.5$, $p = .011$) and 5th days of play ($U = 116$, $p = .003$). RL animals produced significantly more dorsal contacts than the LL on the 2nd ($U = 128.5$, $p = .008$) and 3rd ($U = 97$, $p = .001$) days of play.

3.2. Play suppression

A clear divergence between the line animals was observed for play suppression after cat collar exposure. Surprisingly, LL animals expressed a rapid and robust return to play levels whereas HL animals remained suppressed throughout the testing period. Each of the behavioral indicators of play was examined for each testing day. LL animals pin frequency was higher than that of the RL on the extinction days 1 ($U = 68.5$, $p = .000$) and 8 ($U = 104$, $p = .009$; Fig. 2A). LL animals produced a higher level of pin frequency than the HL animals on the worn cat collar day ($U = 143.5$, $p = .029$) and all of the extinction days (day 1: $U = 76$, $p = .001$; day 2: $U = 110.5$, $p = .014$; day 3: $U = 109$, $p = .013$; day 4: $U = 128.5$, $p = .048$; day 5: $U = 100.5$,

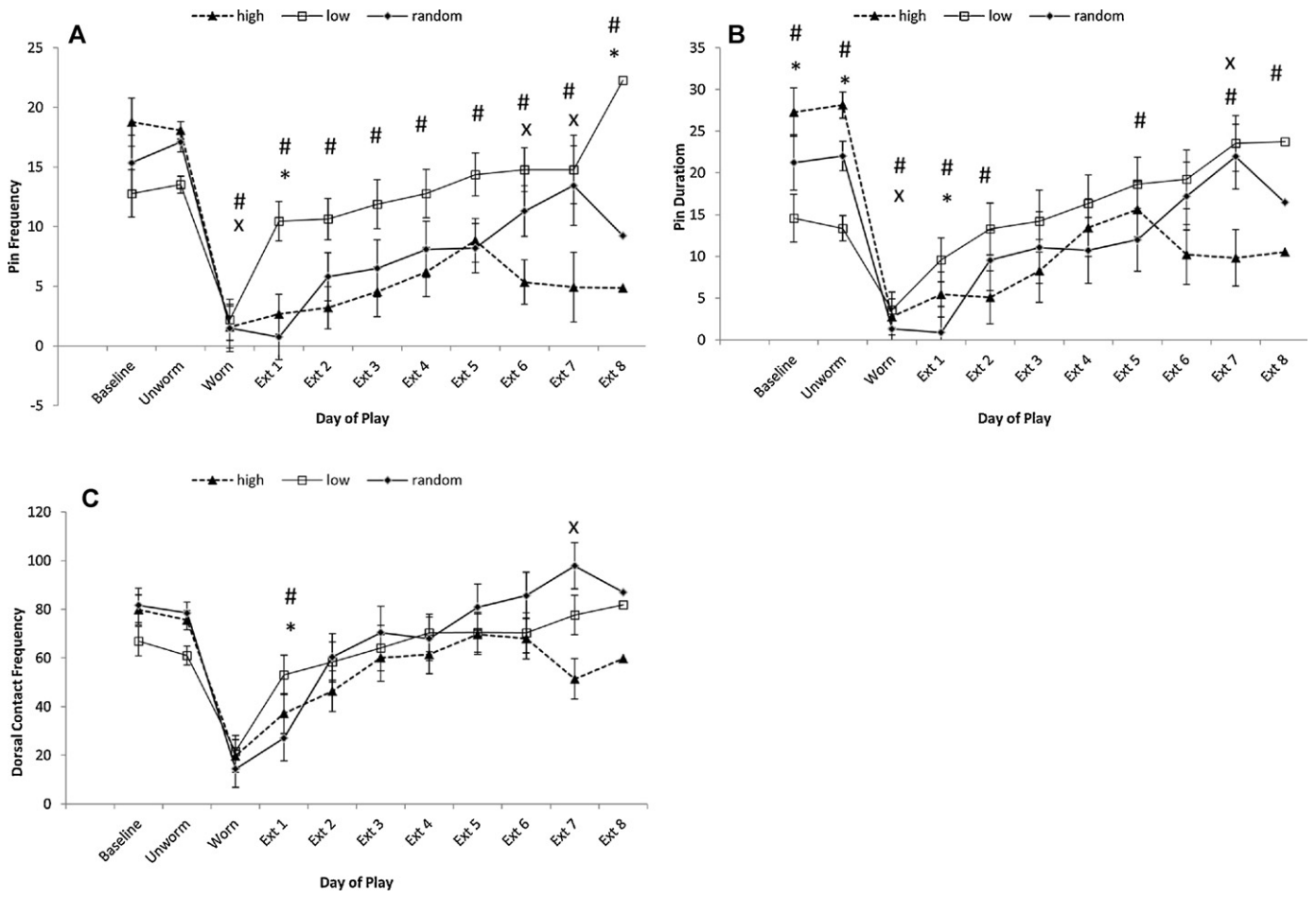

Fig. 2. Play behaviors measured during play suppression. Pin frequency, pin duration and dorsal contact frequency varied significantly within and among the selectively bred lines during the play development experiment. (A) There were no significant differences in pin frequency between the baseline and unworn cat collar day in any of the selectively bred lines of animals ($p > .05$ for all comparisons). All three animal lines showed a significant reduction in pin frequency on the worn cat collar day when compared to baseline levels and levels produced on the unworn cat collar day ($p < .005$ for all comparisons). RL animals showed a significant reduction in levels of pinning on extinction days 1, 2, 3, 5 when compared to baseline levels ($p < .005$ for all comparisons). LL animals return to baseline levels of pinning on the 1st extinction day ($p > .005$). HL animals never return to baseline levels of pinning ($p < .005$ for all comparisons). (B) There were no significant differences in pin duration between the baseline and unworn cat collar day in any of the selectively bred lines of animals ($p > .05$ for all comparisons). All three animal lines showed a significant reduction in pin duration on the worn cat collar day when compared to baseline levels and levels produced on the unworn cat collar day ($p < .005$ for all comparisons). RL animals showed significant reductions in pin duration on extinction days 1 and 2 when compared to baseline levels ($p < .005$ for all comparisons) and returned to baseline on the 3rd day of extinction ($p > .005$). LL animals returned to baseline durations of pinning on the 1st extinction day ($p > .005$) and exceeded this on the 8th day of extinction ($p < .005$). HL animals never return to baseline levels of pin duration ($p < .005$ for all comparisons). (C) There were no significant differences in the frequency of dorsal contacts between the baseline and unworn cat collar day in any of the selectively bred lines of animals ($p > .05$ for all comparisons). All three animal lines showed a significant reduction in dorsal contacts on the worn cat collar day when compared to baseline levels and levels produced on the unworn cat collar day ($p < .005$ for all comparisons). RL animals showed suppressed levels of dorsal contacts on the 1st extinction day ($p < .005$). The LL dorsal contact frequencies returned to baseline levels on the 1st extinction day ($p > .005$) and exceeded baseline levels on the 2nd day of extinction ($p < .005$). HL animals' levels of dorsal contacts remained below baseline until the 3rd extinction day ($p < .005$ for all comparisons) ($^{*}p < .02$, LL versus RL comparisons; $^{\times}p < .02$, HL versus RL comparisons; $^{\#}p < .02$, HL versus LL comparisons).

$p = .007$; day 6: $U = 100$, $p = .007$; day 7: $U = 84$, $p = .002$; day 8: $U = 43$, $p = .000$). RL animals pin frequency was higher than that of the HL on the worn cat collar day ($U = 77$, $p = .000$) and extinction days 6 ($U = 123.5$, $p = .038$) and 7 ($U = 90.5$, $p = .003$).

RL animals' pin duration was longer than that of the HL animals on the worn cat collar day ($U = 77$, $p = .000$) and extinction day 7 ($U = 102$, $p = .007$; Fig. 2B). The RL also produced longer pin duration than the LL on the baseline day of play ($U = 115.5$, $p = .022$), unworn cat collar day ($U = 122.5$, $p = .036$), extinction day 1 ($U = 75.5$, $p = .000$). High line animals produced longer pin durations than the LL animals on the baseline ($U = 107$, $p = .012$) and the unworn cat collar ($U = 84$, $p = .002$) days of play behavior testing. Interestingly, the LL animals produced significantly longer pin durations when compared to the HL on the worn cat collar day ($U = 142.5$, $p = .026$) and extinction days 1, 2, 5, 7 and 8 (day 1: $U = 85$, $p = .001$; day 2: $U = 121$, $p = .029$; day 5; $U = 111$, $p = .016$; day 7: $U = 91.5$, $p = .003$; day 8: $U = 95$, $p = .005$).

LL animals produced significantly higher frequencies of dorsal contacts when compared to the RL and HL animals on the 1st extinction day (LL versus RL: $U = 125$, $p = .042$; LL versus HL: $U = 110$, $p = .015$; Fig. 2C). RL animals produced significantly higher frequencies of dorsal contacts than the high line on the 7th extinction day ($U = 99.5$, $p = .006$).

3.3. Ultrasonic vocalizations during play suppression

RL animals produced more 22 kHz USVs on the worn cat collar day than the LL animals ($U = 160$, $p = .038$; Fig. 3). HL animals also produced more 22 kHz USVs on the worn cat collar day than the LL animals ($U = 160$, $p = .038$). The HL and RL did not differ on 22 kHz USV emission. No higher frequency of 50 kHz USVs was observed for any of the lines during the cat collar exposure session or post-collar sessions.

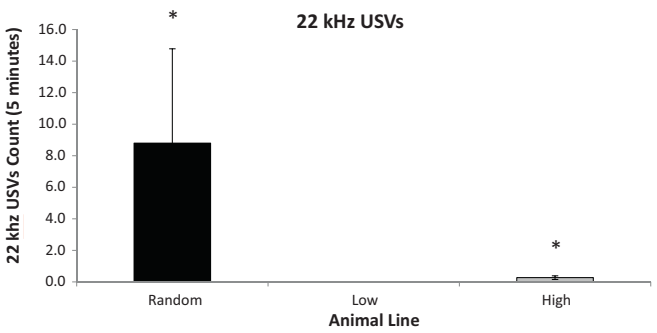

Fig. 3. 22 kHz USV emission on the worn cat collar day. 22 kHz USV emission varied among the selectively bred lines of animals on the worn cat collar day of the play suppression experiment. RL and HL animals produced significantly more than LL animals (*$p < .05$ for all comparisons).

4. Discussion

4.1. Play behavior and ultrasounds in line animals

Overall, selectively bred animals (HL and LL) express an atypical play profile during development and after aversive stimulus exposure. As expected, HL animals expressed higher levels of play relative to LL animals but this was specific to the type of play behavior (dorsal contacts and not pins) and to the context/associative learning history (i.e., earlier rather than later during development and not following aversive stimulus experience). Current results demonstrate that 22 kHz USV emission was higher in the HL rather than LL animals during the cat odor exposure. A difference in emotionality compared to recent work using the same animal lines [26]. Importantly, these same USV signals were not enhanced in HL animals relative to the RL. USV emission in aversive contexts has been shown to be highly variable and dependent upon several factors including: (1) the nature, intensity and duration of the aversive stimulus, (2) the experiences of the animal both in the testing procedure and general living conditions (i.e., social or isolate housing) and (3) the social composition during the testing (i.e., audience effects) [27–32]. We discuss the results related to these factors and others. Clearly, the isolate housing and potent aversive stimulus of predatory odor used in the current study played a role in the types of results obtained, and these factors seem to interact differently in the diverse lines of animals. Additionally, we believe that variation in the strength of associative learning between aversive stimulus and context experienced (i.e., small chamber without an escape location) contributed to the differences among the groups in behavior and ultrasound production. The lack of 50 kHz USV emission during the play suppression experiment is not entirely unexpected. 50 kHz USVs are associated with positive emotional stimuli [3,13,33], and it would be unusual for rats to emit this subtype of USV during an encounter with the scent of a predator. The aversive nature of predatory odor has been shown to produce reductions in 50 kHz USV emission [34].

4.2. Line animals and action competition during play development

Play in rats rapidly increases during the juvenile period and then sharply declines thereafter [25,35]. Play behavior during this time has been shown to be crucial for development of social, emotional and cognitive functions [36,37]. Typical play bouts consist of coordinated sequences of dorsal contacts leading up to pinning [16,38]. The pattern of action is thought to be important for expression of and normal influences from play experiences [38,39]. Dorsal contacts are thought to be appetitive play behaviors that involve social approach and recognition and function as a type of 'tag you're it' type of signal [16] while pins are more related to the consummatory phase of play. The distribution of play actions was altered in line animals with HL animals expressing more dorsal contacts and LL more pins. One possible reason for this difference is that HL animals experience higher levels of positive affect related to anticipation during play bouts. These animals commit more dorsal contacts in order to prolong the bouts and experience greater anticipatory excitement. This possibility fits with previous work showing higher positive affect in HL animals [26].

A second possible reason for the shift in play actions is related to the selection process that used heterospecific play between the animal and experimenter. Since this play experience does not easily allow active pinning by the animal, it could be that the HL animals are ones that selectively prefer play composed of fewer pins and relatively more dorsal contacts. This type of co-selection for targeted (USV production) and non-targeted (dorsal contact expressing) traits has been found in rodent behavioral work [40] and might reflect a natural competition between play actions which is observed in many diverse action groups [41,42]. Future work could examine the influence of different selection strategies (e.g., simulate pinning and being pinned during heterospecific play) on play behavior phenotypes.

4.3. Line animals, play suppression and emotional learning

Suppression of play after cat odor varied substantially between the HL and LL animals. HL animals expressed enhanced suppression while LL animals returned to baseline play more rapidly. The predator odor effect of behavioral inhibition is potent suppressing typical behaviors for an extended period after a single 'cat odor' experience [20,23]. One reason for HL animals displaying enhanced play suppression could be elevated associative learning during the cat odor trial. HL animals may be forming a stronger associative link between the unconditioned stimulus of the cat odor and the conditioned stimulus of the play chamber. When re-experiencing the play chamber, these animals show enhanced fear responsiveness because of the power of the conditioning process. LL animals could have impaired emotional learning and display reduced conditioning upon return to the chamber. Previous work has found altered conditioning processes in HL and LL rat pups [5]. In this study, rat pups were conditioned to prefer an odor with nursing behavior. HL rat pups displayed normal conditioning but LL animals showed a lack of preference for the conditioned cue (lemon scent). How could altered conditioning occur in these two lines of animals? One possibility relies on the selection protocol for choosing the breeders for each of the lines similar to the possible effect on action tendencies (see above). The breeders are chosen following a bout of tickle sessions, one a day for 4 days. The crucial ultrasounds used to delineate potential breeders from non-breeders are tallied on the final day of tickle exposure after the extensive experience with the contextual cues of the test chamber and general procedure. The HL breeders would be animals most likely to associate the cues of the chamber with repeated positive experiences and emit the highest levels of USVs in relation to anticipation and experience of the tickle while LL animals would be the opposite in both anticipation abilities as well as actual affective experience during the test session. This would be another example of co-selection for a process not targeted during the breeding yet the result has a major impact on how the selected lines behave in emotional paradigms.

Hand-in-hand with this idea, enhanced learning could be related to higher intensity emotional states experienced during the cat odor trial. HL animals emitted higher levels of aversive-related USVs during the cat odor trial compared to the LL animals. Despite play suppression during that trial being similar among the groups, this difference could reflect higher levels of negative affect and stress during this situation. If this combines with a higher level of

associative learning capabilities, then the result could be prolonged emotional impact on behavior. Other work has found USV emission to vary dependent on the intensity of the aversive stimulation [43]. This relationship between enhanced fear (dose–response study) and predisposed emotionality should be tested in future work as an animal model to better understand how altered unconditioned responsiveness can interact with enhanced emotional learning.

4.4. Final considerations and implications

Discrepancies between the current work and previous work may be due to differences in the type and intensity of the aversive stimuli used in the present study. Most of the previous work on these line animals focused upon a set of unconditioned emotional responses to stimuli. The present work examined both unconditioned and conditioned responses to emotional stimuli. Additionally, cat odor as an aversive stimulus may tap into a partially different emotional operating system when compared with other aversive stimuli used [16] making the line animals response more instinctual and making the emotional memories more dependent upon these basic systems. These sites could include periaquaductal grey, tegmental regions of the midbrain and reticular formation [44,45]. Key emotional learning sites include amygdala and midline prefrontal cortex [46,47].

The work on developmental profile of play and on play suppression arose from two different sets of related work. The ontogeny work is modeled after earlier work attempting to facilitate play using isolate housing and short play sessions over a number of days [25]. The play suppression paradigm is modeled after a recent study using the cat collars for more precise stimulus control [23]. These two paradigms varied in some ways that might have led the baseline levels of play to differ including: (1) development work was done on alternating days while the play suppression was continuous testing and (2) the developmental work was performed under red light during the light cycle while the play suppression was under white light. These differences could account for relatively higher play behavior in LL animals in experiment 1 versus 2 because of the reduced stress when tested under red light conditions. HL animals may have lower pinning in the same context due to reduced associative learning opportunities when the trials are interspersed with 24 h in-session intervals.

The majority of animal models of emotion focus on emotional learning or regulation [48,49]. A minority focus on primary process emotions using animal models [50]. It is rare for a single model to explore both functions and to examine the possible interactions. These animals hold promise because they have clear alterations in emotional states and display altered emotional learning. This type of model more accurately portrays the type of complex emotional dysfunction observed in mental illnesses and offers a novel way to understand related underlying mechanisms involved.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbr.2012.01.012.

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