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Altered vocal communication in adult vasopressin-deficient Brattleboro rats

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ABSTRACT

The neuropeptide, arginine vasopressin (AVP), has been implicated in social communication across a diverse array of species. Many rodents communicate basic behavioral states with negative versus positive valence through high-pitched vocalizations above the human hearing range (ultrasonic vocalizations; USVs). Previous studies have found that Brattleboro (Bratt) rats, which have a mutation in the *Avp* gene, exhibit deficits in their USVs from the early postnatal period through adolescence, but the magnitude of this effect appears to decrease from the juvenile to adolescent phase. The present study tested whether Bratt rats continue to exhibit USV deficits in adulthood. USVs of adult male and female Bratt and wild type (WT) rats were recorded in two contexts: a novel environment (empty arena) and a social context (arena filled with bedding soiled by same-sex conspecifics). The number, frequency, and duration of 50 kHz USVs were quantified by DeepSqueak after validation with manual scoring. Twenty-two kHz measures were quantified by manual scoring because DeepSqueak failed to accurately detect USVs in this frequency range. Adult Bratt rats did not exhibit deficits in the number of 50 kHz USVs: male Bratt rats emitted similar 50 kHz USVs as male WT rats, whereas female Bratt rats emitted more USVs than female WT rats. USV frequency and duration were altered in adult Bratt rats, but in a context-dependent manner. Twenty-two kHz USVs were less affected by the Bratt mutation. The present study demonstrates how chronic AVP deficiency impacts social communication across the lifespan. The present findings reveal a complex role for AVP in vocal communication, whereby disruption to the *Avp* gene leads to sex-, context-, and developmental phase-specific effects on the quantity and spectrotemporal characteristics of rat USVs.

1. Introduction

Communication is integral to the coordination of social interactions, and lifelong communication deficits can accompany disorders of social development such as autism spectrum disorders [1–4]. Communication occurs through several modalities ranging from behavioral displays, facial expressions, and posture to chemical and vocal signals [5–9]. During social interactions, rats communicate in each of these modalities, including ultrasonic vocalizations (USVs), which are vocalizations at frequencies above the human hearing range. Rat USVs have been divided into three broad categories based on the developmental stage of the animal and the frequency of the USV waveform. Rat pups emit 40 kHz (~30–50 kHz) USVs when separated from their mother. These maternal separation-induced USVs are reduced by anxiolytics and are thought to signal an anxiety-like state of the pup [10–13]. In older rats, behavioral states with negative valence are thought to be communicated by USVs around 22 kHz (~18–30 kHz), whereas behavioral states with positive valence are thought to be communicated by USVs around 50 kHz (~30 kHz–100 kHz; [14–16]). Twenty-two kHz USVs occur in contexts that elicit behavioral displays of anxiety, threat, and distress [17,18]. In contrast, 50 kHz USVs occur in prosocial contexts (e.g., social contact, play, and tickling) and are accompanied by mesolimbic dopamine release [19–22].

The neuropeptide, arginine vasopressin (AVP), regulates many types

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of social behaviors, including behavioral, chemical, and vocal modes of social communication across several species from fish to mammals (for reviews see [23–25]). AVP has been implicated in rat USVs, but most studies have focused on maternal separation-induced USVs of rat pups. In general, AVP facilitates maternal separation-induced USVs [10,13,26,27], although this effect may depend upon site of action (peripheral vs. central; [28]). More recent studies have demonstrated that AVP also facilitates 50 kHz USVs emitted by juveniles engaged in social play [29,30]. To our knowledge, the role of AVP in adult rodent USVs has only been assessed in a single study. In this study, adult female mice lacking the vasopressin 1b receptor (*Avpr1b* null mice) emitted fewer USVs during a resident-intruder test [31]. The frequency of the USV waveform was also affected, with *Avpr1b* null mice emitting USVs with a lower frequency than heterozygous or wild type controls [31].

The Brattleboro (Bratt) rat is a useful model for studying the role of AVP in behavioral development [32–34]. Bratt rats have a natural mutation in the *Avp* gene leading to the loss of endogenous vasopressin across the lifespan [35]. Knockout models, such as the Bratt rat, can determine the consequences of loss of gene function across development on the brain and behavior. The Bratt mutation impacts social behaviors of pups (e.g., aggregation; [36]), juveniles/adolescents (e.g., social play, huddling; [30]), and adults (e.g., social recognition/discrimination, social interactions; [37–39]). Several studies have found that USVs are impacted by the Bratt mutation. Bratt rat pups emit fewer maternal separation-induced USVs [39,40]. Similarly, Bratt juveniles and adolescents emit fewer 50 kHz USVs during social interactions [30]. Notably in this study, the effect in juveniles (postnatal day 34) appeared more robust than that in adolescents (postnatal day 44), suggesting that the impact of the Bratt mutation on USVs might wane across the lifespan. Whether adult Bratt rats also exhibit deficits in USVs has not been previously examined. Hence, the full impact of AVP-deficiency across the lifespan is not known.

The current study tested whether the deficits in USVs of Bratt rats persist into adulthood. Because the type of USVs emitted and the effects of AVP manipulations often depend on environmental context [41,42], rats were tested under two different contexts. USVs of adult male and female wild type and Bratt rats were recorded in a novel environment (empty arena) and a social context (arena with soiled bedding from same-sex animals). The number, duration, and principal frequency of USVs were quantified using DeepSqueak software [43]. To ensure accuracy of automated scoring in our behavioral paradigm, DeepSqueak measures were validated by comparison to manual scoring. To understand the full impact of a gene on behavioral development, adult animals should be assessed. The present study completes the developmental profile of USVs in Bratt rats, providing a more complete understanding of the role of the *Avp* gene in the development of social communication.

2. Materials and methods

2.1. Subjects and housing conditions

Animals used in the current study were adult, aged 3-4 months, male and female wild-type (WT; $n = 13$ male, 12 female) and Brattleboro (Bratt; $n = 13$ male, 12 female) rats. Subjects were offspring from heterozygous (Het) male and Het female breeding pairs from our local breeding colony. This breeding scheme generates WT and Bratt subjects within the same litters. The colony was established from rats purchased from the Rat Resource and Research Center (University of Missouri, Columbia, MO). Prior to the start of testing, subjects were housed in sex-, genotype-, and age-matched groups of 2 to 3 rats per cage in plastic cages (17.32 in x 8.86 in x 8.07 in). Ambient temperature was maintained at 23 °C, and the light:dark cycle was set at 12 h:12 h (lights on at 05:00, EST). Animals were provided with food and water ad libitum. All procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University at Buffalo, State University of New York.

2.2. Experimental procedures

All behavioral testing was conducted during the light phase, starting 3 h after lights on and ending 5 h after lights on. Rats were single housed the day before the first behavioral test (novel environment context) and remained single-housed for the remainder of the experiment. Behavioral tests were conducted inside sound attenuating testing chambers (31.5 in x 38.75 in x 53.5 in) equipped with LED lights and an UltraSoundGate CM16/CMPA microphone capable of recording USVs. All subjects underwent both behavioral testing procedures; rats were first tested in the novel environment context, then in the social context 7–9 days later. Rats were tested in 3 cohorts of 16–18 animals per cohort. Sex and genotype were balanced across each cohort.

2.2.1. Experiment 1: ultrasonic vocalizations in a novel environment

For the novel environment, rats were placed in an open field arena (inner dimensions: 28.5 in x 28.5 in x 16 in) and allowed to explore the novel environment for 5 min (as in [18]). USVs were recorded by a microphone suspended above the arena. After the 5 min had elapsed, animals were placed back into their home cage. The arena was wiped down with 70 % ethanol between each behavior test to remove odors and waste products. One audio file of a female Bratt rat was corrupted during recording, reducing the sample size in this group to 11.

2.2.2. Experiment 2: ultrasonic vocalizations in a social context

For the social context, rats were placed in an arena (inner dimensions: 19.625 in x 19.625 in x 16 in) filled 1.5 inches deep with corn cob bedding (Teklad 7092) for 10 min on 4 consecutive days (as in [44]). The first 3 days served to collect social odors from the cohort of rats (e.g., odors from urine, fecal matter, and skin/fur contact with the bedding). On the fourth day (testing day), USVs were recorded by a microphone suspended above the arena. The bedding was not changed, and the arena was not cleaned until after the 4th behavioral testing day was completed. Between days, the arena was covered with plastic wrap to preserve the build-up of odors from the testing cohort that occurred over the experiment. Separate arenas were used for male and female rats so as not to expose animals to opposite sex odors. After testing was completed for each cohort, the bedding was removed, and arenas were cleaned with 70 % ethanol. Four audio files were corrupted during recording (2 male Bratt rats, 2 female Bratt rats), reducing sample sizes of these groups to 11 male Bratt rats and 10 female Bratt rats.

2.3. Ultrasonic vocalization measures

2.3.1. Ultrasonic vocalization recordings

USVs were recorded using UltraSoundGate CM16/CMPA microphone suspended above the arena. The microphone was connected to a computer in the adjoining room via Avisoft Bioacoustics UltraSoundGate 116Hb. Rodent vocalizations were recorded with the sampling rate set at 250 kHz in 16-bit format; spectrograms were created via fast Fourier transformation (FFT; 256 FFT length, 100 % frame, FlatTop window, and 50 % time window overlap) using SASLab Pro software (Avisoft Bioacoustics).

2.3.2. Ultrasonic vocalization classification and analysis

USVs were classified into two categories based on their principal frequency, 22 kHz or 50 kHz. In the current study, 22 kHz USV calls were defined as having a principal frequency between 15 and 30 kHz; 50 kHz USV calls were defined as having a principal frequency greater than 30 kHz.

USV number, principal frequency, and call length for all audio files were scored manually by a researcher blind to conditions and by DeepSqueak software [43]. For manual scoring, the onset and offset of each call were marked using Avisoft Bioacoustics software. Calls were

marked only if they were a minimum of 5 ms in duration; calls were considered distinct if separated by at least 5 ms. The manually scored principal frequency was calculated by averaging the frequency at onset, offset, and peak amplitude of the call.

DeepSqueak scoring was validated by comparing its output data to the manually scored data. We first identified the range of DeepSqueak confidence thresholds that detected similar numbers of USVs to manual scoring in a subset of 8 audio files for each context (novel environment and social); 9 confidence thresholds were assessed – 40 %, 50 %, 55 %, 57.5 %, 60 %, 62.5 %, 65 %, 67.5 %, and 70 %. For 50 kHz USVs, counts were within 5 % of that detected by manual scoring at the 57.5 % and 60 % thresholds for the novel environment context and at the 62.5 % and 65 % thresholds for the social context. Based on this initial assessment, we selected the 57.5 %, 62.5 %, and 70.0 % confidence thresholds for further validation. The number, principal frequency, and call duration of USVs detected at these thresholds were correlated with those from the manual scoring for all audio files in both contexts. All measures for 50 kHz USVs were highly correlated between DeepSqueak and manual scoring. Overall, the 62.5 % threshold provided the best match to the manually scored data, for which correlation coefficients ranged from 0.883 to 0.997 for all USV measures in both contexts (Supplemental Table 1). We next determined whether the choice of threshold (57.5 %, 62.5 %, or 70 %) affected the statistical findings for all USV measures (number, principal frequency, or call duration). Significant main effects and interactions from the 62.5 % threshold analyses almost exactly matched those from the manual scoring analysis. From these validation procedures, the 62.5 % confidence threshold was deemed the optimal threshold for 50 kHz USVs in the present experiments and was used for all 50 kHz USV analyses and figures reported in the Results section.

The same validation procedures were conducted for 22 kHz USVs. Here, however, DeepSqueak scoring did not match the manually scored data at any threshold. There was poor agreement for the number of 22 kHz USVs detected by manual and DeepSqueak scoring at all thresholds in the initial assessment (greater than 38 % difference for all thresholds between 50 % and 70 %). Furthermore, USV measures (number, principal frequency, and duration) in both contexts (novel environment and social context) obtained from the 57.5 %, 62.5 %, and 70 % DeepSqueak confidence thresholds did not match measures derived from manual scoring; correlation coefficients were low and largely non-significant (Supplemental Table 2). Visual inspection of DeepSqueak and manual scoring indicated that DeepSqueak missed several USVs in the 22 kHz frequency range. Hence, manual scoring was used for all 22 kHz analyses and figures.

2.4. Genotyping procedures

Rats were genotyped using the methodology developed by Paul and colleagues [30]. Ear tissue was collected between postnatal day (P) 13–16, and the DNA was subsequently extracted using REDExtract-N-Amp Tissue PCR Kit (SigmaAldrich). The region surrounding the Bratt mutation was amplified by PCR; forward primer = GACGAGCTGGGCTGCTTC, reverse primer = CCTCAGTCCCCCACTTAGCC. The resulting PCR product was incubated in a 37 °C water bath for 24 h with the restriction endonuclease BCG1 (New England BioLabs), which cuts the mutant Bratt allele in half while leaving the WT allele intact. Samples were then run on a 2 % agarose gel by electrophoresis to visualize the mutant and WT DNA bands. The bisected Bratt allele appears as a single band of ~95 bp, and the WT allele as a single 222 bp band. Het animals contain both the Bratt and WT bands. This genotyping procedure has been validated by DNA sequencing [30] and phenotyping of diabetes insipidus of the Bratt rats by measuring overnight water intake [45].

2.5. Statistical analyses

Effects on USV measures (number, principal frequency, and call duration) were assessed by 2 × 2 between-subjects ANOVAs with genotype (WT vs Bratt) and sex (male vs female) as the independent variables. Significant interactions were further assessed using Tukey's HSD post hoc correction. For the validation of DeepSqueak confidence thresholds significant main effects and post-hoc comparisons were compared between manual scoring and the three confidence thresholds. Additionally, USV number, principal frequency, and call duration detected by each DeepSqueak threshold were correlated with those detected by manual scoring using Pearson's correlation (see Supplemental Materials).

3. Results

3.1. Exp 1: novel environment

Fifty kHz USVs. There was a sex-dependent effect of the Bratt mutation on the number of 50 kHz USV calls in the novel environment (Fig. 1A). The main effect of genotype [$F_{(1,\ 45)} = 26.586$, $p < .001$, ANOVA] and the genotype X sex interaction [$F_{(1,\ 45)} = 9.610$, $p = .003$, ANOVA] were significant. Post hoc analyses revealed that female Bratt rats produced more 50 kHz USVs than female WT rats [$p < .001$, Tukey's HSD], whereas this genotype difference was not significant in males [$p = .445$, Tukey's HSD]. Female Bratt rats also emitted more 50 kHz calls than male Bratt rats [$p = .015$, Tukey's HSD]. Neither sex nor genotype altered the principal frequency of 50 kHz calls (Fig. 1B; main effect of genotype, $F_{(1,\ 45)} = 3.500$, $p = .068$, main effect of sex, $F_{(1,\ 45)} = 1.710$, $p = .198$, sex X genotype interaction, $F_{(1,\ 45)} = 2.094$, $p = .155$, ANOVA). Bratt rats produced longer 50 kHz calls than WT rats (Fig. 1C; main effect of genotype, $F_{(1,\ 45)} = 14.882$, $p < .001$, ANOVA); the main effect of sex and the sex X genotype interaction were not significant [$F_{(1,\ 45)} \leq 0.553$, $p \geq .461$, ANOVA].

Twenty-two kHz USVs. Neither the Bratt mutation nor sex significantly affected the number of 22 kHz calls produced in the novel environment (Fig. 2A; main effect of genotype, $F_{(1,\ 45)} = 3.343$, $p = .074$, main effect of sex, $F_{(1,\ 45)} = 0.681$, $p = .414$, genotype X sex interaction, $F_{(1,\ 45)} = 0.365$, $p = .549$, ANOVA). The principal frequency of 22 kHz calls was higher in Bratt rats relative to WT rats (Fig. 2B; main effect of genotype, $F_{(1,\ 45)} = 47.744$, $p = .022$, ANOVA); the main effect of sex and the sex X genotype interaction were not significant ($F_{(1,\ 45)} \leq 0.241$, $p \geq .626$, ANOVA). Twenty-two kHz call duration was not influenced by either sex or genotype (Fig. 2C; $F_{(1,\ 45)} \leq 1.942$, $p \geq .170$ for main effects of sex and genotype as well as the interaction, ANOVA).

3.2. Exp 2: social context

Fifty kHz USVs. As in the novel environment, there was a sex-dependent effect of the Bratt mutation on the number of 50 kHz USV calls in the social context (Fig. 3A). The main effect of sex, main effect of genotype, and the interaction were all significant ($F_{(1,\ 42)} = 19.443$, $p < .001$, ANOVA, $F_{(1,\ 42)} = 4.726$, $p = .035$, ANOVA, $F_{(1,\ 42)} = 4.505$, $p = .040$, ANOVA, respectively). Post hoc analyses revealed that female Bratt rats produced more 50 kHz USVs than female WT rats ($p = .024$, Tukey's HSD), whereas this genotype difference was not significant in males ($p = 1.00$, Tukey's HSD). Female Bratt rats also emitted more 50 kHz calls than male Bratt rats [$p < .001$, Tukey's HSD]. Unlike in the novel environment, Bratt rats produced 50 kHz calls with lower principal frequencies than WT rats across both sexes (Fig. 3B; main effect of genotype, $F_{(1,\ 42)} = 22.902$, $p < .001$, ANOVA); main effect of sex and the sex X genotype interaction were not significant ($F_{(1,\ 42)} \leq 0.403$, $p \geq .529$, ANOVA). Females produced longer 50 kHz calls than WT rats (Fig. 3C; main effect of sex, $F_{(1,\ 42)} = 9.328$, $p = .004$, ANOVA).

Twenty-two kHz USVs. Neither the Bratt mutation nor sex influenced the number of 22 kHz USVs emitted or their principal frequency in a social context (Fig 4A,B; $F_{(1,\ 42)} \leq 2.271$, $p \geq .139$ for main effects of genotype and sex as well as the interaction, ANOVA). However, Bratt rats emitted longer 22 kHz USV calls than WT rats (Fig. 4C; main effect

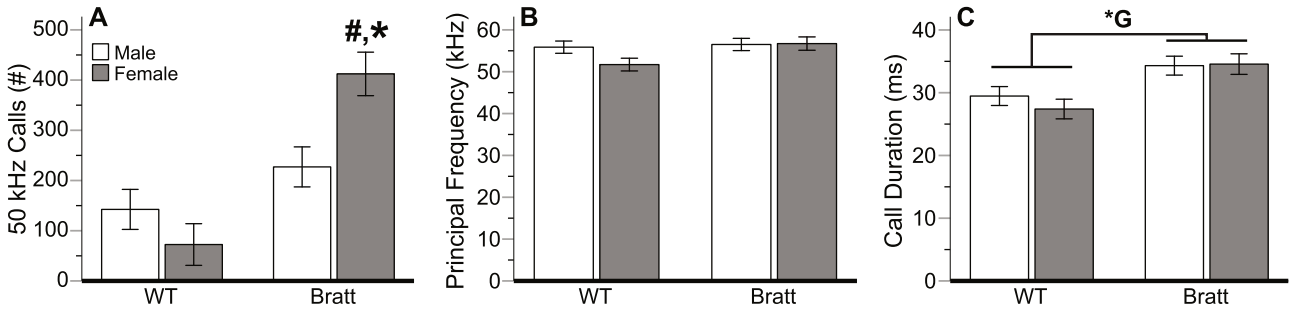

Fig. 1. The Brattleboro mutation alters the quantity and spectrotemporal characteristics of 50 kHz USVs emitted in a novel environment. Mean (± s.e.m.) number (A), principal frequency (B), and duration (C) of 50 kHz USVs emitted by Brattleboro (Bratt) and wild type (WT) rats in a novel empty arena. * Indicates significant genotype difference within sex ($p < .05$, Tukey's HSD). # Indicates significant sex difference within genotype ($p < .05$, Tukey's HSD). *G Indicates significant main effect of genotype ($p < .05$, ANOVA).

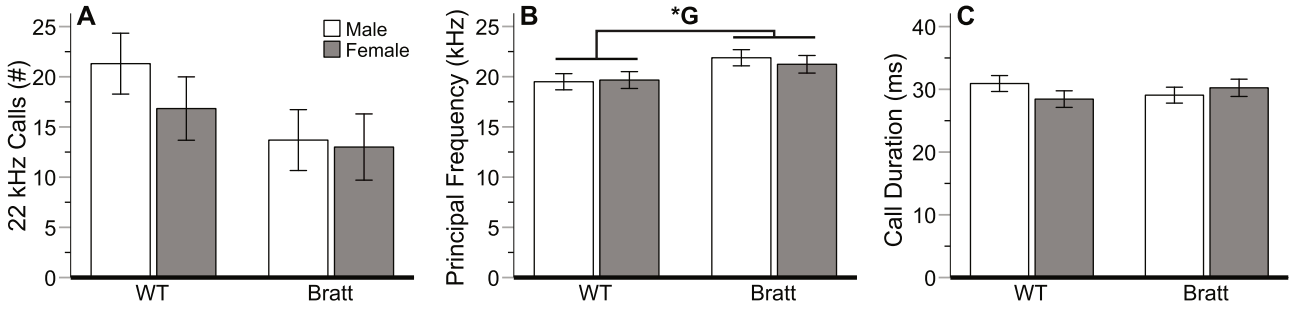

Fig. 2. The Brattleboro mutation alters principal frequency, but not the quantity or call duration, of 22 kHz USVs emitted in a novel environment. Mean (± s.e.m.) number (A), principal frequency (B), and duration (C) of 22 kHz USVs emitted by Bratt and WT rats in a novel empty arena. *G Indicates significant main effect of genotype ($p < .05$, ANOVA). Abbreviations defined in Fig. 1.

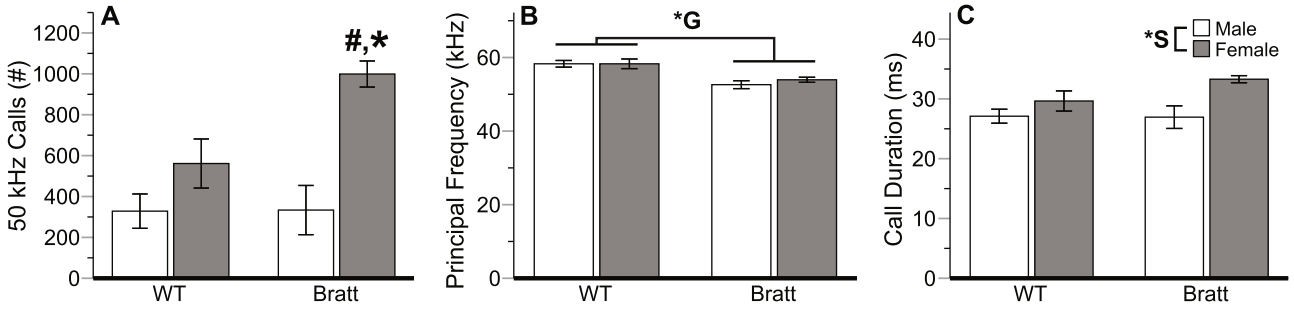

Fig. 3. The Brattleboro mutation alters the quantity and spectrotemporal characteristics of 50 kHz USVs emitted in a social context. Mean (± s.e.m.) number (A), principal frequency (B), and duration (C) of 50 kHz USVs emitted by Bratt and WT rats in an arena filled with soiled bedding. * Indicates significant genotype difference within sex ($p < .05$, Tukey's HSD). # Indicates significant sex difference within genotype ($p < .05$, Tukey's HSD). *G Indicates significant main effect of genotype ($p < .05$, ANOVA). *S Indicates significant main effect of sex ($p < .05$, ANOVA). Abbreviations defined in Fig. 1.

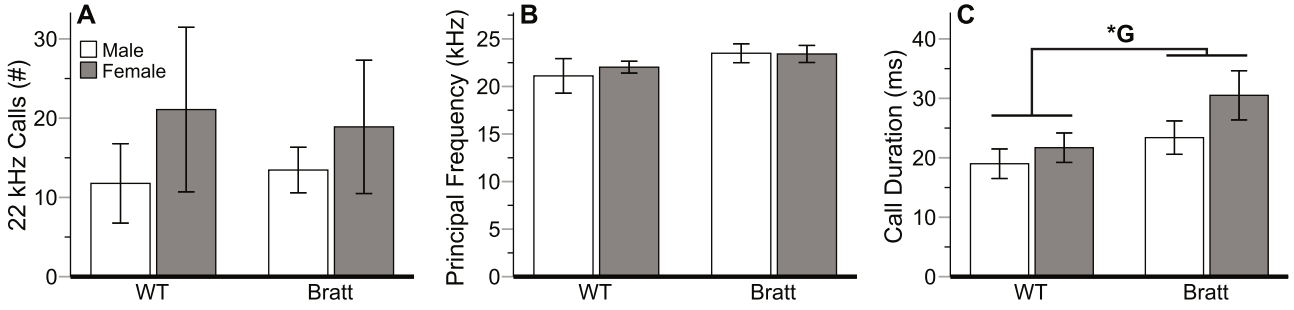

Fig. 4. The Brattleboro mutation alters the call duration, but not the quantity or principal frequency, of 22 kHz USVs emitted in a social context. Mean (± s.e.m.) number (A), principal frequency (B), and duration (C) of 22 kHz USVs emitted by Bratt and WT rats in an arena filled with soiled bedding. *G Indicates significant main effect of genotype ($p < .05$, ANOVA). Abbreviations defined in Fig. 1.

of genotype, $F_{(1, 42)} = 4.950$, $p = .032$, ANOVA); main effect of sex and the sex X genotype interaction were not significant ($F_{(1, 42)} \leq 2.737$, $p \geq .106$, ANOVA).

4. Discussion

The current study investigated the impact of lifelong AVP deficiency on vocal communication of adult rats. The quantity and spectrotemporal characteristics of USVs emitted by adult male and female Bratt rats were compared to those of WT rats in two contexts – a novel environment (novel context) and a familiar arena with soiled bedding (social context). The Bratt mutation led to a sex-dependent increase in the number of 50 kHz USVs: female, but not male, Bratt rats emitted more 50 kHz calls than their WT counterparts. Spectrotemporal characteristics of the 50 kHz USVs were also impacted, but in a context-dependent manner: Bratt rats emitted 50 kHz USVs with a lower mean principal frequency in the social context only, whereas Bratt 50 kHz call durations were longer in the novel environment only. The Bratt mutation did not alter the number 22 kHz USVs, but did affect their spectrotemporal characteristics, again in a context dependent manner. Caution is warranted when interpreting the 22 kHz data given the low number of 22 kHz calls emitted by both genotypes under both testing contexts. Overall, these findings suggest a complex role for AVP in adult rat vocal communication that differs depending on the sex of the animal, the context in which the animal is tested, and the developmental phase of the animal (see below).

In contrast to the present findings, prior work has found that Bratt rat pups, juveniles, and adolescents of both sexes emit fewer 50 kHz calls [30,39,40]. Hence, there appears to be a shift in the Bratt phenotype from decreased USVs early in development to either no deficit (males) or increased USVs (females) as Bratt rats reach adulthood. This fits with the observation that USV deficits were less robust in adolescent compared to juvenile Bratt rats [30]. Intracerebroventricular administration of a vasopressin 1a receptor (V1aR) antagonist decreases 50 kHz USVs in juvenile rats [29], indicating a facilitatory role for AVP during the juvenile phase. The developmental shift in USV phenotype of adult Bratt rats could reflect a change in the role of AVP in USV production across adolescence or compensatory mechanisms in response to the absence of AVP in late adolescence. To our knowledge, the effects of pharmacological AVP manipulations on USVs have not been conducted in adult rats. Hence, at present, we cannot distinguish between these two possibilities. Alternatively, the sex-dependent increase in adult Bratt USVs could be due to different testing conditions – pups were tested in a maternal-separation paradigm [39,40], juveniles/adolescents in a full social interaction paradigm [30], and adults in novel environment and soiled bedding paradigms (present study). Given the similar results of adult Bratt rats in the two testing paradigms in the present study, we feel this possibility is less likely. The similar decreases seen for Bratt pups and juveniles/adolescents in two markedly different testing paradigms is also not consistent with a context-dependent effect of AVP on USV quantity. Siberian hamsters (and likely other rodents) emit more spontaneous USVs during their active phase [46], raising the possibility that time of testing could impact results of USV experiments. However, time of testing does not appear to impact behavioral tests that elicit social behaviors, at least in mice [47]. Bratt USV experiments were conducted in the light phase (pups and adults [39,40, present study]) and dark phase (juveniles; [30]), but time of testing does not match the pattern of results (USV deficits in pups and juveniles versus sex-dependent increases in adult females).

Spectrotemporal characteristics are thought to play important communicative roles. In rats, broad differences in spectrotemporal frequency are thought to reflect different behavioral states – 50 kHz USVs reflect those with positive valence, whereas 22 kHz USVs reflect those with negative valence [14–18]. Mice rely predominantly on frequency to distinguish between tone categories [48]. Pup USV call frequency and duration are altered in several mouse models of ASD [49], and playback studies have found that altered pup USVs in a rodent model of autism are less effective at triggering retrieval responses from the mother [50]. The Bratt mutation impacts USV spectrotemporal characteristics indicating that their calls sound different than their WT counterparts. When tested in a social context, juvenile, adolescent, and adult Bratt rats of both sexes as well as adult female *Avpr1b* null mice emit 50 kHz USVs with lower principal frequencies [30,31, present study]. The present study was the first to test USVs of Bratt rats in a non-social context, and we found that USV frequency was not affected by the Bratt mutation in the novel empty arena. This suggests that AVP's effects on this parameter are specific to social situations. It is important to note that there were other methodological differences between the two contexts in addition to the presence of social odors, including the duration of isolation (1 day versus 8–10 days), presence of bedding, and size of the arena. Hence, further experiments are needed to test the hypothesis that AVP's actions on USV frequency are specific to social contexts. Effects of genetic AVP manipulations on USV call duration are less consistent. No difference was seen in adult female *Avpr1b* null mice [31]. In juvenile Bratt rats, effects are seen in a minority of USV subtypes, and the direction of the effect differs across these subtypes [30]. In the present study, 50 kHz USV call duration of Bratt rats was increased in the novel context, but not the social context. Collectively, these findings suggest a more subtle, nuanced role for AVP in the modulation of call duration.

Effects of the Bratt mutation on 22 kHz USVs were limited to spectrotemporal characteristics, and here too, effects were context dependent. As seen in juvenile and adolescent rats [30], the Bratt mutation did not alter the number of 22 kHz USVs emitted during testing. Although this could suggest that AVP deficiency has a greater impact on positive compared to negative behavioral states, this conclusion is premature. Twenty-two kHz USVs thought to reflect aversive behavioral states typically have a long duration 300–1200 ms [51,52]. In contrast, 22 kHz USVs in the present experiment were ~20–30 ms. Brudzynski et al. [53] identified 2 distinct populations of 22 kHz USVs with differing durations – short calls that were 20–200 ms long and long calls that were 300 to >2000 ms long. The 22 kHz calls of the present experiment fall within the short call group, the functional significance of which is not known. Hence, it is not clear if the short 22 kHz calls in the present experiment reflect behavioral states with negative valence.

AVP plays an important role in behavioral sex differences. The sex difference in the AVP pathway originating from cells in the bed nucleus of the stria terminalis and medial amygdala is one of the most consistently detected sex differences across species, with males exhibiting more cells and fiber projections than females [54,55]. AVP manipulations, particularly those targeting this pathway, often lead to different effects in males and females [56,57]. Hence, it is important to study the effects of AVP manipulations in both sexes. In the present experiment, there was a sex difference in the number of 50 kHz USVs in Bratt rats (females > males) that was not present in WT rats. This suggests that the presence of AVP prevents the emergence of sex differences in USV quantity. The idea that AVP acts to decrease some behavioral sex differences has been proposed previously [58]. Notably, sex differences were not reported for USV quantity of juvenile Bratt rats [30], raising the possibility that the rise in gonadal hormones at puberty contribute to the sex difference in Bratt rats. Only a few studies have assessed the effects of gonadal hormones on USVs. In this limited literature, testosterone has been reported to increase and estradiol decrease USVs emitted in response to social, sexual, or drug stimuli [59–61]. Given that the number of 50 kHz USVs in the present study was higher in female Bratt rats, it is interesting to speculate that estradiol's suppression of USVs in females and/or testosterone's facilitation of USVs in males may require the presence of AVP. More studies are needed to better understand how gonadal steroids influence USVs and whether AVP modulates or mediates their actions.

Automated USV analysis is becoming increasingly popular as it provides several advantages over manual scoring including faster analysis and reduced bias [62]. Nevertheless, it is important to validate the

scoring procedures. Factors such as type of arena, presence of bedding, and species could affect the accuracy of the analysis or the settings required for accurate analysis. In the current experiment we validated the DeepSqueak scoring procedures for our setup and rats by comparing USV measures and analysis obtained from several different DeepSqueak confidence thresholds to those obtained from manual scoring. Notably, while there was high agreement between DeepSqueak and manual scoring for the 50 kHz USVs, DeepSqueak did not accurately detect 22 kHz USVs at any confidence threshold. In the present study, there was more background noise in the lower frequency range which may have made detection of 22 kHz USVs more difficult. This was true regardless of whether bedding was or was not used in the testing arena. Adjustments to the arena that dampen noises from scratching and impact with the plastic arena might be needed to increase accuracy in this frequency range. This highlights the need to validate automated procedures for each testing setup.

There are several caveats that must be considered when using a "conventional" knockout model, such as the Bratt rat, where the gene has been disrupted across all tissues throughout life. As discussed above, compensatory mechanisms can occur during development in response to the loss of gene function. Hence, it is important to consider findings from conventional knockout models using a developmental or lifespan perspective, where effects could be due to direct actions of the gene product or indirect compensatory actions resulting from the loss of the gene product during development. USV findings in Bratt pups and juveniles/adolescents are similar to those seen after administration of AVP antagonists [10,26,27,30,31,63], indicating the early-life Bratt USV phenotype reflects directs actions of AVP at the time of behavioral assessment. Whether this is also true for the shift in USV phenotype in adult Bratt rats is not yet clear.

We also cannot determine whether the present findings are due to the loss of AVP action in the brain or periphery. AVP can act in several peripheral tissues including blood vessels and heart, and it has been proposed that AVP's actions in the periphery could provide feedback to the brain regarding behavioral state [64]. Furthermore, the absence of AVP in Bratt rats disrupts water reabsorption at the level of the kidney, leading to excessive drinking and urination (i.e. diabetes insipidus; [65]). Effects of the Bratt mutation on anxiety-like and depressive-like behaviors persist after restoration of AVP's actions on the kidney with a peripheral agonist indicating that anxiety-like and depressive-like phenotypes of Bratt rats are not a side effect of the diabetes insipidus [66]. Similar studies are needed to rule out this possibility for USVs.

Manipulating AVP neural circuits affects USVs in adult mice, consistent with a neural site of action. Knockdown of AVP expression in the BNST or administration of a V1aR antagonist at one of its target projections, the lateral habenula, decrease sociosexual USVs of male, but not female, adult mice [67,68]. These findings do not match the female-specific augmentation of USVs seen in adult Bratt rats, but questions remain about the precise role of this pathway in mice. BNST AVP knockdown only affects USVs toward other males and not females, whereas administration of the V1aR antagonist in the lateral habenula only impacts USVs toward females and not males. Furthermore, genetic ablation of AVP cells in the BNST does not alter USVs of male or female mice toward either sex [69]. The BNST and MeA are thought to act in concert to regulate social behavior. Hence it is possible that AVP must be disrupted in both brain areas simultaneously to elicit more consistent and robust actions. Genetic ablation of AVP cells in the paraventricular nucleus of the hypothalamus (PVN) also does not impact male or female mouse USVs [70]. Given that the PVN and supraoptic nucleus often act in concert, here too it may be necessary to ablate cells in both nuclei.

AVP has been implicated in social communication across a diverse array of species, from fish to humans [e.g., 28,31,71–76]. Disruptions in AVP have also been noted in autism spectrum disorders, a collection of neurodevelopmental disorders characterized by deficits in social interactions and social communication. Concentrations of AVP in the cerebral spinal fluid (CSF) are lower in children with ASD compared to neurotypical controls [77]. Notably, this reduced CSF AVP is present in newborns, well before the onset of behavioral symptoms [78]. These findings indicate that AVP function is altered very early in the pathogenesis of ASD. Specific variants of DNA segments within the vasopressin receptor 1a gene are also associated with ASD diagnosis, raising the possibility that these variants confer vulnerability to developing ASD [79–81]. AVP treatment has shown promise for improving social function in children with ASD [82]. The Bratt rat is a useful model to study the impact of lifelong disruptions to AVP and may provide insight into how AVP deficiency contributes to social deficits. As seen in mouse models of ASD, Bratt rats exhibit altered USVs early on in development [10,30,39,49]. Few models, however, have characterized the full developmental USV profile. The present study indicates that USV deficits early in life do not necessarily persist into adulthood. It will be important to determine the mechanism responsible for this developmental shift and whether this is a common feature in rodent models of mental health disorders.

5. Conclusions

AVP has been implicated in a diverse array of social behaviors across many species [23–25]. Studies using the Bratt rat have shown that lifelong AVP deficiency impacts behavioral states and social behaviors, including altered vocal communication [30,36–40,83,84]. The present experiment reveals a complex role for AVP in vocal communication, whereby disruption to the *Avp* gene leads to sex-, context-, and developmental phase-specific effects on the quantity and spectrotemporal characteristics of rat USVs. These findings demonstrate how chronic AVP deficiency impacts social communication across the lifespan.

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CRediT authorship contribution statement

Chloe N. Cordes: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Cole P. Fredericks:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Linging Liu:** Writing – review & editing, Investigation. **Destiny J. Brakey:** Writing – review & editing, Investigation. **Derek Daniels:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Matthew J. Paul:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors have no competing interests to declare.

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Supplementary materials

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