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Long-term hyperglycaemia induces qualitative and quantitative changes in rat ultrasonic vocalizations

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Diabetes mellitus (DM) is currently considered a global epidemic. Recently, the relation between diabetes and mood disorders has been established, as well as a link with altered behavioural traits, that may depend on changes in the emotional state. To further elucidate the interplay between DM and changes in the emotional state, we evaluated in long-term diabetic hyperglycaemic (HG) or in control (CTRL) male rats the emission of ultrasonic vocalizations (USVs), a behavioural marker that may express the emotional states of rats and their changes. Rats were exposed to a conspecific matched for condition (i.e. HG or CTRL), to elicit calling behaviour; then, the numbers and acoustic parameters (duration, maximum frequency, minimum frequency, bandwidth) were scored for the 50-kHz USVs (appetitive) and 22-kHz USVs (aversive) emitted. Dyads of HG rats emitted higher numbers of 50-kHz USVs, compared with dyads of CTRL rats. Moreover, differences in acoustic parameters were detected between the 50-kHz USVs emitted by HG and CTRL rats. These effects were observed for the total 50-kHz USVs emitted, as well as for the flat and frequency modulated categories of 50-kHz calls. Finally, HG rats (30% of dyads) but not CTRL rats also emitted 22-kHz USVs. These findings demonstrate that long-term diabetic hyperglycaemia causes qualitative and quantitative changes in ultrasonic calling behaviour in male rats. Moreover, they suggest that measuring the emission of USVs may become an informative approach to study the effects of hyperglycaemia in rat models of DM.

Keywords 22-kHz calls, 50-kHz calls, Affect, Aversion, Flat calls, Frequency modulated calls, Reward

Diabetes mellitus (DM) is a common chronic metabolic disorder with a worldwide increasing prevalence[1–3]. DM comprises a group of heterogeneous disorders characterized by an increase in blood glucose concentrations resulting from defects in insulin secretion, insulin action, or both[4,5]. DM stems from the progressive failure of the body to manage circulating blood glucose, resulting in a wide array of comorbidities, from traditional micro- and macrovascular complications, such as stroke, nephropathies, retinopathies, and peripheral neuropathies, to recently acknowledged liver diseases[6,7]. Recent evidence also indicates that DM is associated with complications affecting the central nervous system. For example, DM has lately emerged as a risk factor for neurodegenerative diseases[8–11]. Moreover, DM can affect superior brain domains (e.g., cognition) and can possibly alter the emotional state. This is suggested by the evidence that diabetic patients may display behavioural abnormalities, such as heightened aggressivity and agitation[12,13], that may depend on underlying alterations in the emotional state[14], often occurring in patients with chronic illnesses such as DM[12,13,15].

Progress in understanding how DM affects the emotional state could come from studies in animal models that allow investigating the effects of long-term hyperglycaemia on neurocircuits that regulate reward and aversion, as well as the behavioural correlates of those effects. Indeed, earlier studies in rodent models of DM have demonstrated the manifestation of altered responses in the elevated plus maze and the forced swim test[16–20], two experimental paradigms classically used to study in rodents the presence of anxious/depressive-like phenotypes that may stem from the presence of a negative emotional state (reviewed in[21]. Moreover, a recent study of our group has demonstrated the occurrence of altered social behaviour in rats exposed to long-term (i.e., 5 months) diabetic hyperglycaemia, which could possibly depend on the presence of modifications in the emotional state[22].

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Indeed, in rats an interplay may exist between social behaviour and emotional states, as demonstrated by other data showing differences in social approach behaviour and in aggressivity towards conspecifics in rats with low or high levels of anxiety, as well as in putative rat models of depression[23–28]. To further investigate the effects that long-term hyperglycaemia has on the emotional state, we deemed interesting to evaluate the emission of ultrasonic vocalizations (USVs) in our model of long-term (i.e., 5 months) HG rats. In fact, USVs are regarded as a behavioural marker that may express the state of arousal, motivation, as well as the presence of positive or negative emotional states in rats[29].

USVs emitted by adolescent/adult rats can be broadly categorized in two families, the "22-kHz USVs" and the "50-kHz USVs", based on their average frequency and supposed behavioural significance[30,31]. The so-called "22-kHz USVs" possess low maximum frequency (up to 35 kHz), long duration (more than 300 ms), and lack significant frequency modulation[32]. Rats emit 22-kHz USVs in response to aversive stimuli; accordingly, these calls are considered behavioural markers of negative emotional states[33]. Conversely, the so-called "50-kHz USVs" possess high maximum frequency (up to 120 kHz), short duration (less than 150 ms) and may display substantial frequency modulation within individual calls[32]. Rats emit 50-kHz USVs in response to appetitive stimuli; accordingly, these calls are considered behavioural markers of positive emotional states[34–36]. On these bases, evaluating the emission of USVs could provide valuable information on the possible changes affecting the emotional state in rat models of DM. Measuring the emission of USVs allows disclosing the occurrence of both positive and negative emotional states of rats, as well as the fluctuation between and intensity of emotional states[37,38]. Conversely, other experimental paradigms that have been so far used to characterize the behavioural phenotype of rat models of DM, such as the elevated plus maze and forced swim test, allow disclosing only behavioural responses that may be related to negative emotional states. Therefore, measuring the emission of USVs as a behavioural marker is a valuable approach to increase the amount of information that can be obtained in preclinical studies about the changes affecting emotional states in experimental models of central and peripheral diseases[21], including models of chronic diseases as DM.

The current study aimed at providing a qualitative and quantitative characterization of the USVs emitted by long-term (i.e., 5 months) HG male rats. The experimental model we used here was selected based on the results of our previous investigations demonstrating that long-term male HG rats display neurodegenerative phenomena affecting the dopaminergic system, as well as abnormalities in motor and social behaviours[22,39]. Accordingly, we aimed to ascertain whether long-term hyperglycaemia could also alter a behavioural response (i.e., the emission of USVs) that is thought to straightforwardly communicate the emotional state of rats[38]. The emission of USVs was elicited by the exposure to a same-sex conspecific matched for condition (i.e. HG or control, CTRL), a procedure that is commonly used to elicit calling behaviour of rats in studies that investigate the qualitative and quantitative parameters of USVs[40–43]. The numbers and acoustic parameters (duration, maximum frequency, minimum frequency, bandwidth) of the 22-kHz USVs and 50-kHz USVs recorded were then quantified. The analysis of 50-kHz USVs included the total number of calls as well as the categories of "flat" calls (i.e., USVs that are thought to serve mainly a function of social coordination) and "frequency modulated" (FM) calls (i.e., USVs that are thought to preferentially express the presence of a positive emotional state)[44].

Materials and methods

Animals

A total of 36 male Sprague-Dawley rats (Charles River, St-Constant, Canada) were used. Rats weighed 175–200 g at the beginning of experiments (i.e., induction of hyperglycaemia) and were housed two per cage under a 12 h light/dark cycle. Standard rodent chow 5075 (Charles River) and water were available *ad libitum*, except during USV recordings. Experiments were carried out in accordance with legislation of the Canadian Council on Animal Care (CCAC) and the study is reported in accordance with ARRIVE guidelines. These experiments were also approved by the Animal Care Committee of the Université du Québec (Trois-Rivières, Canada), protocol number MGM-5-14. Accordingly with the reduction policy of the CCAC (Council on Animal Care), these animals were shared with a previous study (Renaud et al. 2024), to maximize the information gained per animal. In that previous study rats received neither pharmacological treatments nor experimental manipulations (i.e., exposure to stress) that could have had an enduring influence on the emission of USVs. Moreover, earlier investigations of our group found no effects of previous experimental use on the emission of USVs in male rats that were: (i) tested in dyads and previously subjected to multiple pairings with different same-sex conspecifics[43]; (ii) tested individually during two different memory tasks that were performed in a counterbalanced order[45]. Accordingly, we may conclude that sharing the animals with a previous investigation is unlikely to have affected the results of the present study.

Induction of hyperglycaemia

Rats were randomly assigned to the CTRL group (n = 16) or HG group (n = 20). For the induction of hyperglycaemia, fasted rats of the HG group received an intraperitoneal (i.p.) injection of nicotinamide (100 mg/kg b.w.) (Millipore Sigma, Oakville, Ont. Canada), followed by a single intraperitoneal injection of the toxin streptozotocin (55 mg/kg b.w.), (Enzo Life Sciences, Farmingdale, NY, USA) targeting pancreatic β cells, as already described[39]. Pre-treatment with nicotinamide was performed to restrain the death of insulin-producing pancreatic β cells and engender a long-term state of hyperglycaemia not requiring glycaemia-lowering treatments[39,46,47]. CTRL rats received two i.p. injections of vehicle (physiological saline). Hyperglycaemia occurred 9 days after injections and only rats with a random glycaemia always above 10mM were included in the HG group. Glycaemia was estimated twice a week for 5 months by collecting 100 µl of blood from the tail vein using a digital glucose meter and matching strips (One Touch Ultra) as already reported[22,39]. No anesthetic agents were used in the present study, since the withdrawal of small amounts of blood from the tail is a non-invasive procedure that does not require anesthesia.

Recording and analysis of ultrasonic vocalizations

Recording of USVs was performed precisely 5 months after the induction of hyperglycaemia. Two unacquainted rats paired for condition (CTRL, 8 dyads; HG, 10 dyads) were placed in a Plexiglas cylinder (diameter, 25 cm; height, 30 cm) with fresh bedding. The cylinder was surrounded by cardboard walls and topped with a lid endowed with an ultrasonic microphone (CM16/CMPA, Avisoft, Berlin, Germany) placed at an average distance of 25 cm from rats. The microphone was connected to an ultrasound-recording device (UltraSoundGate 116 Hb, Avisoft, Berlin, Germany) and constant gain was maintained throughout recordings. USV recordings lasted 10 min, a timeframe selected according to our previous studies assessing the emission of USVs in dyads of unacquainted rats[42,43].

The software SASLab Pro 4.52 (Avisoft, Berlin, Germany) was used to convert USV recordings into spectrograms with the following settings: 512 FFT-length, Hamming window and 75% overlap frame set-up[48]. Afterwards, three experienced experimenters, blind to the glycaemic conditions of rats, inspected and manually cleaned all signals that could not be unambiguously classified as USVs. The SASLab Pro 4.52 software was then used to count the numbers of 22-kHz and 50-kHz USVs, defined according to the criteria previously described (see Introduction and[32]). Since USV analysis is a labour-intensive procedure[49,50], the analysis was limited to the calls emitted every other minute of recording for a total of 5 min analyzed. Moreover, the values of duration, minimum frequency, maximum frequency, and bandwidth (i.e., maximum frequency minus minimum frequency) were calculated for the individual calls analyzed. Finally, the 50-kHz USVs identified were further categorized according to their bandwidth in flat (i.e., calls with a bandwidth lower than 5000 Hz) and FM (i.e., calls with a bandwidth higher than 5000 Hz), as previously described[51,52].

Statistical analysis

Means ± S.E.M. were calculated for: (i) the numbers of 22-kHz USVs; (ii) the numbers of 50-kHz USVs (total and categorized); (iii) the values of acoustic parameters (bandwidth, duration, maximum frequency, minimum frequency) emitted by each dyad of rats. One dyad of rats did not emit flat USVs and that dyad was excluded from the statistical analysis of flat calls only. Normality and homoscedasticity of data were tested with Kolmogorov-Smirnov and Levene tests. For normally distributed data, group differences were analyzed with *t*-test. Welch's correction was applied if data did not show homoscedasticity. For non-normally distributed data, group differences were analyzed with the two-sample Kolmogorov-Smirnov test. The ROUT method (Q = 1%) was used to search for potential outliers, and no outliers were identified in the data collected. Statistical analyses were performed with Prism 8 (GraphPad, La Jolla, CA, USA) for Windows. Levene test was performed with an online calculator (https://www.socscistatistics.com/tests/). Group differences were considered statistically significant at $p < 0.05$.

Results

Emission of 50-kHz ultrasonic vocalizations

Number of calls

Dyads of HG rats emitted higher numbers of 50-kHz USVs when placed inside the test cage compared with dyads of CTRL rats (Fig. 1A), as shown by Welch's *t*-test (t = 2.505, df = 11.37, $p = 0.0286$). Moreover, dyads of HG rats emitted higher numbers of flat calls (Fig. 1B) when placed inside the test cage compared with dyads of CTRL rats, as shown by two-sample Kolmogorov-Smirnov test (D = 0.6750, $p = 0.0207$). Finally, dyads of HG rats emitted higher numbers of FM calls (Fig. 1C) when placed inside the test cage compared with dyads of CTRL rats, as shown by Welch's *t*-test (t = 2.328, df = 11.65, $p = 0.0388$).

Duration of calls

Dyads of HG rats emitted 50-kHz USVs of longer duration compared with calls produced by dyads of CTRL rats (Fig. 2A), as shown by Welch's *t*-test (t = 3.208, df = 12.47, $p = 0.0072$). Moreover, dyads of HG rats emitted flat calls of longer duration compared with calls produced by dyads of CTRL rats (Fig. 2B), as shown by *t*-test (t = 4.625, df = 15, $p = 0.003$). Finally, dyads of HG rats emitted FM calls of longer duration compared with calls produced by dyads of CTRL rats (Fig. 2C), as shown by Welch's *t*-test (t = 3.148, df = 12.20, $p = 0.0083$).

Minimum frequency of calls

Dyads of HG rats emitted 50-kHz USVs with a lower minimum frequency compared with calls produced by dyads of CTRL rats (Fig. 3A), as shown by *t*-test (t = 4.092, df = 16, $p = 0.009$). Moreover, dyads of HG rats emitted flat calls with a lower minimum frequency compared with calls produced by dyads of CTRL rats (Fig. 3B), as shown by *t*-test (t = 4.679, df = 15, $p = 0.003$). Finally, dyads of HG rats emitted FM calls with a lower minimum frequency compared with calls produced by dyads of CTRL rats (Fig. 3C), as shown by *t*-test (t = 3.779, df = 16, $p = 0.0016$).

Maximum frequency of calls

Dyads of HG rats emitted 50-kHz USVs with a lower maximum frequency compared with calls produced by dyads of CTRL rats (Fig. 4A), as shown by Welch's *t*-test (t = 2.376, df = 12.19, $p = 0.0347$). Moreover, dyads of HG rats emitted flat calls with a lower maximum frequency compared with calls produced by dyads of CTRL rats (Fig. 4B), as shown by *t*-test (t = 4.667, df = 15, $p = 0.003$). Finally, dyads of HG rats emitted FM calls that had a trend towards a lower maximum frequency compared with calls produced by dyads of CTRL rats (Fig. 4C). However, this effect did not reach statistical significance, as shown by *t*-test (t = 1.618, df = 16, $p = 0.1251$).

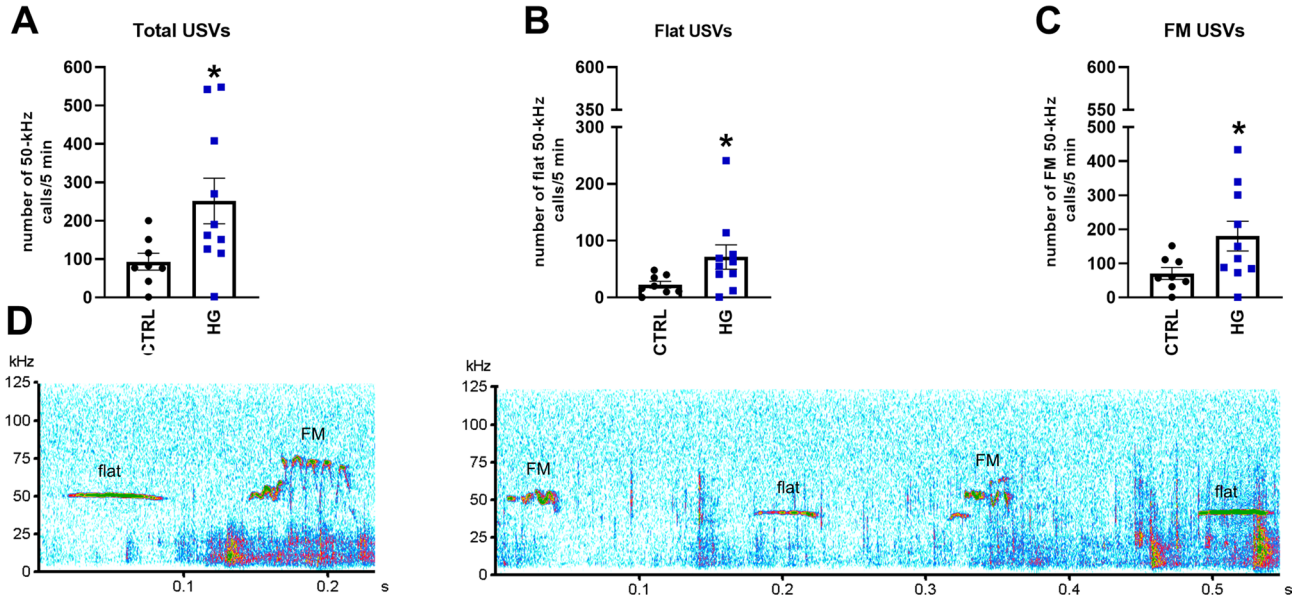

Fig. 1. Numbers of 50-kHz USVs emitted by dyads of CTRL and HG rats. The figure reveals the total number of 50-kHz USVs (**A**) and the numbers of flat (**B**) and frequency modulated (**C**) 50-kHz USVs emitted by dyads of rats. Panel D illustrates examples of 50-kHz USVs recorded in the present study. * $p < 0.05$, compared with CTRL rats. $N = 8$ dyads for CTRL rats; $N = 10$ dyads for HG rats. CTRL = control; FM = frequency modulated; HG = hyperglycaemic; USVs = ultrasonic vocalizations.

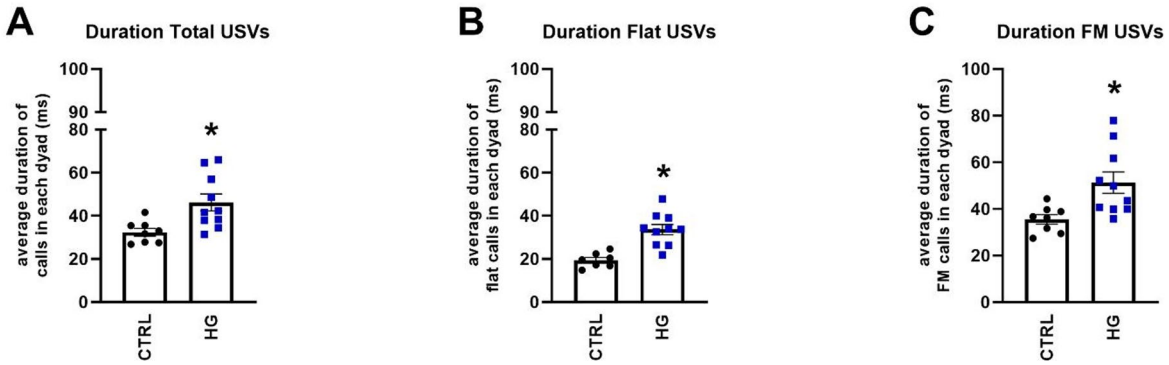

Fig. 2. Duration of the 50-kHz USVs emitted by dyads of CTRL and HG rats. The figure shows the average duration, calculated for each dyad of rats, of the total number of 50-kHz USVs (**A**) and of the flat (**B**) and the frequency modulated (**C**) 50-kHz USVs analyzed. * $p < 0.05$, compared with CTRL rats. $N = 8$ dyads for CTRL rats; $N = 10$ dyads for HG rats. CTRL = control; FM = frequency modulated; HG = hyperglycaemic; USVs = ultrasonic vocalizations.

Bandwidth of calls

Dyads of HG rats emitted FM 50-kHz USVs with a broader bandwidth compared with calls produced by dyads of CTRL rats (Fig. 5C), as shown by two-sample Kolmogorov-Smironov test ($D = 0.6500$, $p = 0.0303$). Conversely, when the total (*t*-test; $t = 0.5375$, $df = 16$, $p = 0.5983$) and flat (*t*-test; $t = 1.301$, $df = 15$, $p = 0.5983$) 50-kHz USVs were considered, no significant changes in call bandwidth were observed between dyads of HG and CTRL rats (Fig. 5A, B).

Emission of 22-kHz ultrasonic vocalizations

The emission of 22-kHz USVs was observed only in 3 dyads of HG rats, and no emission of 22-kHz USVs occurred in CTRL rats. Dyads of HG rats emitted "typical" 22-kHz USVs (i.e., duration > 300 ms, maximum frequency < 32 kHz), as well as 22-kHz USVs that were either preceded or followed by signals in the 50-kHz band of calls. The numbers and acoustic parameters of the 22-kHz USVs analyzed are described in Table 1. Figure 6 shows examples of 22-kHz USVs recorded in the present study.

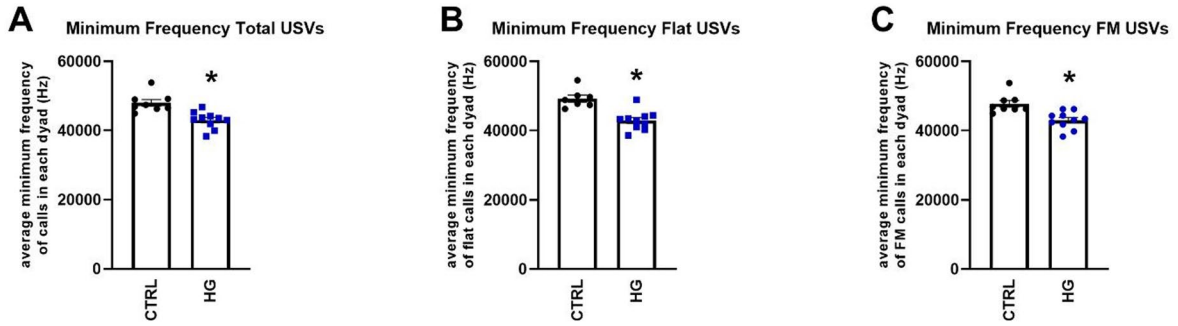

Fig. 3. Minimum frequency of the 50-kHz USVs emitted by dyads of CTRL and HG rats. The figure displays the average minimum frequency, calculated for each dyad of rats, of the total number of 50-kHz USVs (**A**) and of the flat (**B**) and the frequency modulated (**C**) 50-kHz USVs analyzed. * $p < 0.05$, compared with CTRL rats. $N = 8$ dyads for CTRL rats; $N = 10$ dyads for HG rats. CTRL = control; FM = frequency modulated; HG = hyperglycaemic; USVs = ultrasonic vocalizations.

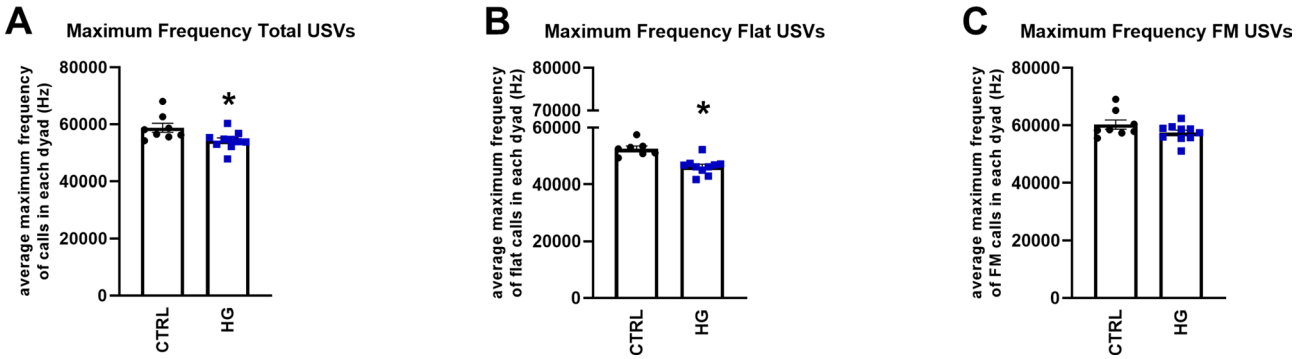

Fig. 4. Maximum frequency of the 50-kHz USVs emitted by dyads of CTRL and HG rats. The figure shows the average maximum frequency, calculated for each dyad of rats, of the total number of 50-kHz USVs (**A**), and of the flat (**B**) and the frequency modulated (**C**) 50-kHz USVs analyzed. * $p < 0.05$, compared with CTRL rats. $N = 8$ dyads for CTRL rats; $N = 10$ dyads for HG rats. CTRL = control; FM = frequency modulated; HG = hyperglycaemic; USVs = ultrasonic vocalizations.

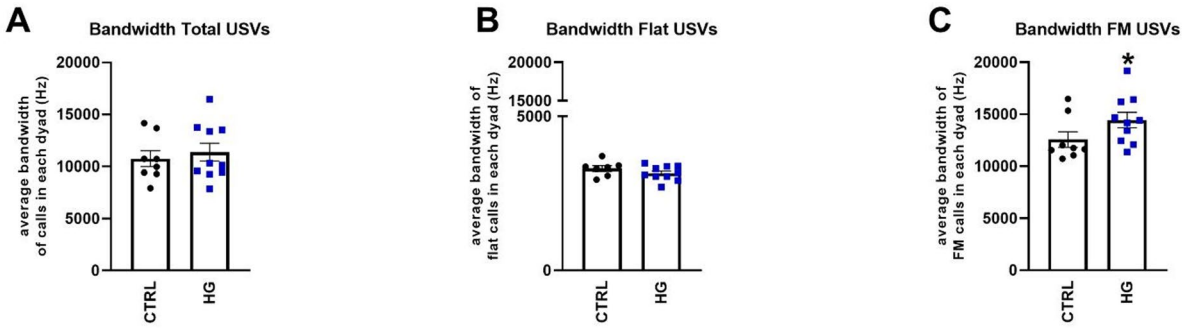

Fig. 5. Bandwidth of the 50-kHz USVs emitted by dyads of CTRL and HG rats. The figure displays the average bandwidth, calculated for each dyad of rats, of the total of 50-kHz USVs (**A**), and of the flat (**B**) and the frequency modulated (**C**) 50-kHz USVs analyzed. * $p < 0.05$, compared with CTRL rats. $N = 8$ dyads for CTRL rats; $N = 10$ dyads for HG rats. CTRL = control; FM = frequency modulated; HG = hyperglycaemic; USVs = ultrasonic vocalizations.

Discussion

Although awareness of the effects of DM on superior brain domains and emotional state has been on the rise lately, further research is warranted in this respect. A significant contribution may come from investigations in animal models of DM, which allow determining the effects of hyperglycaemia on neurocircuits that regulate

	Total Number	Average number per dyad	Average duration per dyad (ms)	Average minimum frequency per dyad (Hz)	Average maximum frequency per dyad (Hz)	Average bandwidth per dyad (Hz)
Typical 22-kHz USVs	106	35.33±12.55	804.91±129.23	19949.09± 525.72	24173.69± 849.39	4210.24± 398.63
22-kHz USVs with 50-kHz components	72	24±8.96	597.94±57.23	21660.35±684.93	42442.89±2932.73	20782.54±1711.91

Table 1. Numbers and acoustic parameters of 22-kHz USVs analyzed in the present study. USVs = ultrasonic vocalization.

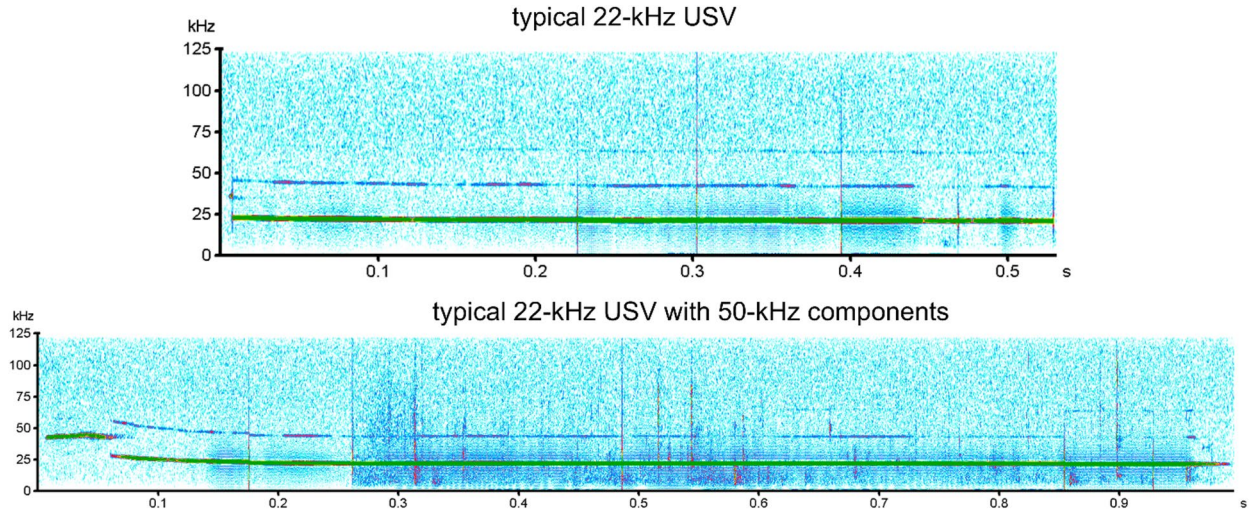

Fig. 6. Examples of 22-kHz USVs recorded in the present study. USV = ultrasonic vocalization.

emotional states, and disclosing how these effects alter behaviour. However, earlier studies in rodents have only partially characterized the effects of diabetic hyperglycaemia on emotional states, possibly because of the use of: (i) paradigms that poorly reflected the chronicity of the diabetic condition, and (ii) behavioural readouts that most effectively capture the presence of negative rather than positive emotional states[16–20].

In the current study, we used an experimental rat model of long-term (i.e., 5-months) hyperglycaemia, where we have previously demonstrated the presence of abnormalities in affiliative/exploratory and aggression-related behaviours, possibly depending on an underlying alteration in the emotional state[22]. Consistent with this view, we found that long-term HG male rats displayed a heightened emission of 50-kHz USVs, a behavioural marker of positive emotional states[44], when evaluated in dyads of unacquainted subjects, compared with CTRL rats. In the same experimental conditions, an increased emission of 22-kHz USVs, a behavioural marker of negative emotional states[33], was observed in a small part (3 dyads on a total of 10 dyads) of the HG rats evaluated. Accordingly, the heightened calling behaviour we observed here may indicate that long-term diabetic hyperglycaemia alters the emotional state by increasing both positive and negative affect in rats.

The emission of 50-kHz USVs is initiated by the activation of the dopaminergic mesocorticolimbic system, particularly in the shell of the nucleus accumbens (NAc)[53,54]. In this regard, it is noteworthy that we have demonstrated in the same model of long-term HG rats used in the present study a significant degeneration of dopaminergic nuclei in the midbrain[39], namely the substantia nigra *pars compacta* and the ventral tegmental area, the latter projecting to the NAc shell. We and others have also found that an increased emission of 50-kHz USVs may occur in dopamine-denervated rats in response to either non-pharmacological or pharmacological stimuli[55–58]. A persistent denervation of dopaminergic systems may result in the instatement of dopamine receptor supersensitivity in striatal and cortical regions[59–61]. Hence, increased responsiveness of dopamine receptors in long-term HG rats could result in a heightened emission of 50-kHz USVs, consistent with the current results. Moreover, the neurodegeneration of dopaminergic systems occurring in our model of long-term diabetic hyperglycaemia may result in a decrease in tonic dopamine transmission in the striatum[39]. Tonic dopaminergic firing consists in the slow and irregular neurotransmitter release generated by the pacemaker activity of mesencephalic neurons, that maintains a baseline level of dopamine in the striatum[62]. When striatal dopamine tonicity is low, phasic dopamine release becomes more relevant. On the other hand, phasic firing consists in a stimulus-mediated discharge of spikes in a short amount of time[62], which raises the levels of striatal dopamine above the baseline, acting as a signal that directs attention to a salient stimulus. When produced in response to a salient stimulus, like the exposure to a conspecific, phasic firing might cause inappropriately intense reactions[63]. Therefore, the possible imbalance in tonic-phasic striatal dopamine transmission may be another mechanism explaining the heightened emission of 50-kHz USVs observed in our model of long-term HG rats. It may be speculated that the increased calling behaviour observed in our long-term HG rats also depended, at

least in part, on the presence of functional/structural changes affecting non-dopaminergic neurotransmitter pathways, that are known to regulate the emission of USVs[38,42,64–67]. However, further studies are warranted to confirm this hypothesis, since the effects of long-term hyperglycaemia on non-dopaminergic transmitter pathways in the rat brain are still undefined.

Analysis of categorized 50-kHz USVs revealed that long-term HG rats emitted higher numbers of both FM and flat calls, compared with CTRL rats. The increased emission of FM 50-kHz USVs suggests that long-term diabetic hyperglycaemia is associated with an increase in positive affect, since FM calls are envisioned as the category of 50-kHz USVs more closely related to the presence of positive emotional states in rats[68]. Conversely, emission of flat 50-kHz USVs is generally considered a way for rats to coordinate social contacts[69]. Since the emission of USVs may also express the state of arousal[29], the increased emission of flat 50-kHz USVs in long-term HG rats may be consistent with a heightened arousal for social stimuli in these animals[22]. However, it cannot be ruled out that the heightened emission of flat calls somehow reflects the presence of changes in the emotional state of long-term HG rats. Indeed, previous studies have demonstrated that the psychostimulant amphetamine, which possesses rewarding properties, significantly elevates the emission of flat 50-kHz USVs in rats[34,48,52,66,70,71]. Conversely, another study has suggested that rats may emit flat 50-kHz USVs to indicate the presence of a negative emotional state of mild intensity[72]. Thus, the increased emission of flat 50-kHz USVs observed in this study could also signal the presence of negative affect in long-term HG rats. This hypothesis may also be consistent with our observations showing that some long-term HG rats emitted 22-kHz USVs, considered a marker of negative emotional state[33].

In this study, long-term diabetic hyperglycaemia affected the acoustic parameters of the 50-kHz USVs emitted by rats. When considered globally, the calls emitted by HG rats displayed longer duration as well as reduced minimum and maximum frequency, but no significant changes in bandwidth, compared with the calls emitted by CTRL rats. Similar changes were observed in the acoustic parameters of flat and FM 50-kHz USVs, although the bandwidth of FM calls was found to be broader in HG rats compared with CTRL rats. These changes in the acoustic parameters of 50-kHz USVs could be a direct consequence of the toxic effects of hyperglycaemia on dopamine transmission[39]. Indeed, earlier investigations have demonstrated that the acoustic parameters of 50-kHz USVs are altered in experimental rat models of dopaminergic hypofunction/neurodegeneration, with a reduction in the values of bandwidth, duration and maximum frequency of calls being generally observed[55,57,73]. These alterations are thought to depend on a deterioration of the laryngeal mechanisms that mediate the production of calls after dopaminergic hypofunction[74,75]. However, it is noteworthy that the modifications in the acoustic parameters of 50-kHz USVs observed in our experimental model of diabetes overlap only partially with those previously reported in models of dopaminergic hypofunction/neurodegeneration, since the calls emitted had increased, rather than decreased, values of duration and, for FM calls only, bandwidth. Accordingly, we hypothesise that changes in the acoustic parameters of the 50-kHz USVs produced by long-term hyperglycaemic rats could either: (i) reflect the presence of different modifications in laryngeal function compared to those occurring in rat models of dopaminergic hypofunction; (ii) or reflect an original behavioural significance, yet to be determined.

By demonstrating the presence of quantitative and qualitative changes in the emission of USVs in long-term HG rats, the present study proposes that measuring ultrasonic calling behaviour may be a pertinent and original approach to investigate how hyperglycaemia influences the emotional state in rat models of diabetes. Nevertheless, the present study has some potential limitations:

1. we observed variability in the numbers of USVs emitted by HG and CTRL rats, similar to what previously reported in rats exposed to either non-pharmacological or pharmacological stimuli[34,43,66,69,76].
2. it has been speculated that the emission of USVs in rats may not be solely a behavioural marker of emotional states[77–79]. For example, studies in rats treated with psychoactive drugs have suggested that the emission of 50-kHz USVs may reflect, in part, the occurrence of phenomena unrelated to the drugs' affective properties[79], such as the presence of overactivation/supersensitivity of dopamine transmission[58,71,80–82]. Importantly, supersensitivity of striatal dopamine receptors may be caused by the degeneration of dopaminergic nuclei in the midbrain[59–61], which we have previously demonstrated in the model of long-term hyperglycaemia used in the present study[39]. Moreover, it may be speculated that the alterations in calling behaviour observed in long-term HG rats depend, at least in part, on the noxious effects that hyperglycaemia has on peripheral nerves and organs. For example, peripheral neuropathy has been demonstrated in rodent models of diabetic hyperglycaemia[19,83]; hence, pain and loss of sensation could influence calling behaviour of HG rats. Previous studies have documented the emission of USVs in experimental models of pain (reviewed in[77]). However, it remains disputed whether ultrasonic calling behaviour is a correlate of the physical intensity of pain or rather reflects the emotional component of pain[84–87]. Furthermore, ear damage and hearing dysfunction have been reported in rodent models of diabetes[88,89]. A recent study has demonstrated that hearing loss may affect the emission of USVs in adult rats, by showing a transient reduction in calling behaviour in deafened rats that were tested in dyads[90]. However, in the present study we observed an increased, rather than a decreased, emission of USVs in dyads of HG rats. This finding suggests that further investigation is needed to clarify the link between ear damage and emission of USVs in rat models of diabetes.
3. Measuring the emission of USVs alone provides only a partial index of affect[79]. Therefore, further investigations in rat models of diabetes are warranted that simultaneously evaluate the emission of USVs and the changes in other behaviours (e.g., maze exploration, immobility) that are classically evaluated as a measure of altered emotional states. These future investigations should also evaluate the effects that phar-

macological treatments with glucose-lowering and/or psychoactive drugs may have on calling behaviour in rat models of DM.

4. It has yet to be precisely determined how specific categories of 50-kHz USVs and changes in the acoustic parameters of calls communicate the modifications in the emotional state or, more in general, the homeostatic state of rats.
5. Finally, the present study was performed only in male animals exposed to a single type of stimulus (i.e., pairing with a same-sex conspecific). Thus, we cannot rule out the possibility that the effects of long-term hyperglycaemia on the emission of USVs may depend, at least in part, on the biological sex of rats and/or on the stimulus used to elicit calling behaviour (for example, pharmacological stimuli; see point 3).

Conclusions

The current study demonstrates the existence of a heightened emission of USVs stimulated by the exposure to a same-sex conspecific and of modifications in the acoustic parameters of calls in long-term HG male rats. Since changes in the emission of USVs may reflect the presence of alterations in the emotional state of rats, these results propose that measuring the emission of USVs may become a relevant approach for studies using rat models of diabetic hyperglycaemia to investigate, at the preclinical level, the changes in the emotional state associated with diabetes or other metabolic diseases.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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References

1. Zimmet, P., Alberti, K. G. M. M. & Shaw, J. Global and societal implications of the diabetes epidemic. *Nature* **414**, 782–787. https://doi.org/10.1038/414782a (2001).
2. Zimmet, P., Alberti, K. G., Magliano, D. J. & Bennett, P. H. Diabetes mellitus statistics on prevalence and mortality: Facts and fallacies. *Nat. Rev. Endocrinol.* **12**, 616–622. https://doi.org/10.1038/nrendo.2016.105 (2016).
3. Lovic, D. et al. The growing epidemic of diabetes mellitus. *Curr. Vasc Pharmacol.* **18**, 104–109. https://doi.org/10.2174/1570161117666190405165911 (2019).
4. Diagnosis and classification of diabetes mellitus. In. *Diabetes Care* https://doi.org/10.2337/dc11-S062 (2011).
5. Cloete, L. Diabetes mellitus: an overview of the types, symptoms, complications and management. *Nurs. Stand.* **37**, 61–66. https://doi.org/10.7748/ns.2021.e11709 (2022).
6. Gregg, E. W., Sattar, N. & Ali, M. K. The changing face of diabetes complications. *Lancet Diabetes Endocrinol.* **4** https://doi.org/10.1016/S2213-8587(16)30010-9 (2016). :537 – 47.
7. Tomic, D., Shaw, J. E. & Magliano, D. J. The burden and risks of emerging complications of diabetes mellitus. *Nat. Rev. Endocrinol.* **18**, 525–539. https://doi.org/10.1038/s41574-022-00690-7 (2022).
8. Pagano, G. et al. Diabetes mellitus and Parkinson disease. *Neurology* **90**, e1654–e1662. https://doi.org/10.1212/WNL.0000000000005475 (2018).
9. Sergi, D., Renaud, J., Simola, N. & Martinoli, M. G. Diabetes, a contemporary risk for Parkinson's disease: Epidemiological and cellular evidences. *Front. Aging Neurosci.* **11**, 302. https://doi.org/10.3389/fnagi.2019.00302 (2019).
10. Burillo, J. et al. Insulin resistance and diabetes mellitus in Alzheimer's disease. *Cells* **10**, 1236. https://doi.org/10.3390/cells10051236 (2021).
11. Athauda, D. et al. The Impact of Type 2 Diabetes in Parkinson's Disease. *Mov. Disord.* **37**, 1612–1623. https://doi.org/10.1002/mds.29122 (2022).
12. Tilov, B. et al. Study of the relationship between aggression and chronic diseases (diabetes and hypertension). *J. Eval Clin. Pract.* **22**, 421–424. https://doi.org/10.1111/jep.12502 (2016).
13. Andrade do N, C. J. & C de AD Alves Relationship between bullying and type 1 diabetes mellitus in children and adolescents: a systematic review. *J. Pediatr. (Rio J).* **95**, 509–518. https://doi.org/10.1016/j.jped.2018.10.003 (2019).
14. Hood, S. & Amir, S. Biological clocks and rhythms of anger and aggression. *Front. Behav. Neurosci.* **12**, 4. https://doi.org/10.3389/fnbeh.2018.00004 (2018).
15. Hysing, M., Elgen, I., Gillberg, C. & Lundervold, A. J. Emotional and behavioural problems in subgroups of children with chronic illness: results from a large- scale population study. *Child. Care Health Dev.* **35**, 527–533. https://doi.org/10.1111/j.1365-2214.2009.00967.x (2009).
16. Meehan, W. P., Leedom, L. J., Nagayama, T. & Zeidler, A. Agonistic behavior patterns in mice with streptozotocin-induced diabetes mellitus. *Physiol. Behav.* **38**, 301–306. https://doi.org/10.1016/0031-9384(86)90098-3 (1986).
17. Hilakivi-Clarke, L. A., Wozniak, K. M., Durcan, M. J. & Linnoila, M. Behavior of streptozotocin-diabetic mice in tests of exploration, locomotion, anxiety, depression and aggression. *Physiol. Behav.* **48**, 429–433. https://doi.org/10.1016/0031-9384(90)90339-6 (1990).
18. Caliskan, H. et al. Aerobic exercise has an anxiolytic effect on streptozotocin–induced diabetic rats. *Acta Neurobiol. Exp.* **80**, 245–255 (2020).
19. Gasparin, A. T. et al. Bixin attenuates mechanical allodynia, anxious and depressive-like behaviors associated with experimental diabetes counteracting oxidative stress and glycated hemoglobin. *Brain Res.* **1767**, 147557. https://doi.org/10.1016/j.brainres.2021.147557 (2021).
20. Wang, H. et al. Enriched environment alleviates NLRP3 inflammasome mediated neuroinflammation in diabetes complicated with depression rats. *Sci. Rep.* **15**, 14214. https://doi.org/10.1038/s41598-025-98312-5 (2025).
21. Simola, N. Experimental psychopharmacology. In: Neuropsychopharmacotherapy. (2022). https://doi.org/10.1007/978-3-030-62059-2_3
22. Renaud, J. et al. Long-term diabetic hyperglycaemia modifies social behaviour in rats. *Endocr. Metabolic Sci.* **16**, 100197. https://doi.org/10.1016/j.endmts.2024.100197 (2024).
23. Henniger, M. S. et al. Unconditioned anxiety and social behaviour in two rat lines selectively bred for high and low anxiety-related behaviour. *Behav. Brain Res.* **111**, 153–163. https://doi.org/10.1016/s0166-4328(00)00151-0 (2000).
24. Malkesman, O. et al. Two different putative genetic animal models of childhood depression. *Biol. Psychiatry*. **59**, 17–23. https://doi.org/10.1016/j.biopsych.2005.05.039 (2006).

25. Beiderbeck, D. I. et al. High and abnormal forms of aggression in rats with extremes in trait anxiety–involvement of the dopamine system in the nucleus accumbens. *Psychoneuroendocrinology* **37**, 1969–1980. https://doi.org/10.1016/j.psyneuen.2012.04.011 (2012).
26. Lukas, M. & Wöhr, M. Endogenous vasopressin, innate anxiety, and the emission of pro-social 50-kHz ultrasonic vocalizations during social play behavior in juvenile rats. *Psychoneuroendocrinology* **56**, 35–44. https://doi.org/10.1016/j.psyneuen.2015.03.005 (2015).
27. Martinez, A. R., Brunelli, S. A. & Zimmerberg, B. Communal nesting exerts epigenetic influences on affective and social behaviors in rats selectively bred for an infantile trait. *Physiol. Behav.* **139**, 97–103. https://doi.org/10.1016/j.physbeh.2014.11.007 (2015).
28. Burke, C. J. et al. A naturalistic method to test depression: Anticipation of play. *Behav. Brain Res.* **398**, 112975. https://doi.org/10.1016/j.bbr.2020.112975 (2021).
29. Premoli, M. et al. Mouse and rat ultrasonic vocalizations in neuroscience and neuropharmacology: State of the art and future applications. *Eur. J. Neurosci.* **57**, 2062–2096. https://doi.org/10.1111/ejn.15957 (2023).
30. Wöhr, M. & Schwarting, R. K. W. Affective communication in rodents: Ultrasonic vocalizations as a tool for research on emotion and motivation. *Cell. Tissue Res.* **354**, 81–97. https://doi.org/10.1007/s00441-013-1607-9 (2013).
31. Bartsoen, E. & Wöhr, M. Detailed spectrographic analysis of rat ultrasonic vocalizations emitted during the acoustic startle response test. *Physiol. Behav.* **270**, 114290. https://doi.org/10.1016/j.physbeh.2023.114290 (2023).
32. Simola, N. & Brudzynski, S. M. Repertoire and biological function of ultrasonic vocalizations in adolescent and adult rats. *Handb. Behav. Neurosci.* https://doi.org/10.1016/B978-0-12-809600-0.00017-2 (2018a).
33. Brudzynski, S. M. Emission of 22 kHz vocalizations in rats as an evolutionary equivalent of human crying: Relationship to depression. *Behav. Brain Res.* **363**, 1–12. https://doi.org/10.1016/j.bbr.2019.01.033 (2019).
34. Pereira, M., Andreatini, R., Schwarting, R. K. W. & Brenes, J. C. Amphetamine-induced appetitive 50-kHz calls in rats: A marker of affect in mania? *Psychopharmacology* **231**, 2567–2577. https://doi.org/10.1007/s00213-013-3413-1 (2014).
35. Wendler, E. et al. Mania-like elevated mood in rats: Enhanced 50-kHz ultrasonic vocalizations after sleep deprivation. *Prog Neuropsychopharmacol. Biol. Psychiatry*. **88**, 142–150. https://doi.org/10.1016/j.pnpbp.2018.07.002 (2019).
36. Burgdorf, J. S., Brudzynski, S. M. & Moskal, J. R. Using rat ultrasonic vocalization to study the neurobiology of emotion: from basic science to the development of novel therapeutics for affective disorders. *Curr. Opin. Neurobiol.* **60**, 192–200. https://doi.org/10.1016/j.conb.2019.12.008 (2020).
37. Brudzynski, S. M. Principles of rat communication: quantitative parameters of ultrasonic calls in rats. *Behav. Genet.* **35**, 85–92. https://doi.org/10.1007/s10519-004-0858-3 (2005).
38. Brudzynski, S. M. Ethotransmission: Communication of emotional states through ultrasonic vocalization in rats. *Curr. Opin. Neurobiol.* **23**, 310–317. https://doi.org/10.1016/j.conb.2013.01.014 (2013).
39. Renaud, J. et al. Dopaminergic neurodegeneration in a rat model of long-term hyperglycemia: preferential degeneration of the nigrostriatal motor pathway. *Neurobiol. Aging.* **69**, 117–128. https://doi.org/10.1016/j.neurobiolaging.2018.05.010 (2018).
40. Willey, A. R., Varlinskaya, E. I. & Spear, L. P. Social interactions and 50 kHz ultrasonic vocalizations in adolescent and adult rats. *Behav. Brain Res.* **202**, 122–129. https://doi.org/10.1016/j.bbr.2009.03.025 (2009).
41. Kisko, T. M. et al. Are 50-kHz calls used as play signals in the playful interactions of rats? II. Evidence from the effects of devocalization. Behav Proc 111:25–33. (2015). https://doi.org/10.1016/j.beproc.2014.11.011
42. Simola, N., Costa, G. & Morelli, M. Activation of adenosine A2A receptors suppresses the emission of pro-social and drug-stimulated 50-kHz ultrasonic vocalizations in rats: Possible relevance to reward and motivation. *Psychopharmacology* **233**, 507–519. https://doi.org/10.1007/s00213-015-4130-8 (2016).
43. Simola, N. et al. Modulation of Rat 50-kHz ultrasonic vocalizations by glucocorticoid signaling: Possible relevance to reward and motivation. *Int. J. Neuropsychopharmacol.* **21**, 73–83. https://doi.org/10.1093/ijnp/pyx106 (2018).
44. Simola, N. & Brudzynski, S. M. Rat 50-kHz ultrasonic vocalizations as a tool in studying neurochemical mechanisms that regulate positive emotional states. *J. Neurosci. Methods*. **310**, 33–44. https://doi.org/10.1016/j.jneumeth.2018.06.018 (2018b).
45. Costa, G., Serra, M. & Simola, N. Association between novel object recognition/spontaneous alternation behavior and emission of ultrasonic vocalizations in rats: Possible relevance to the study of memory. *Brain Sci.* **11**, 1053. https://doi.org/10.3390/brainsci11081053 (2021).
46. Szkudelski, T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. *Exp. Biol. Med.* **237**, 481–490. https://doi.org/10.1258/ebm.2012.011372 (2012).
47. Badole, S. L. et al. Cardioprotective activity of pongamia pinnata in streptozotocin-nicotinamide induced diabetic rats. *Biomed. Res. Int.* **2015**, 403291. https://doi.org/10.1155/2015/403291 (2015).
48. Simola, N. et al. Pharmacological characterization of 50-kHz ultrasonic vocalizations in rats: Comparison of the effects of different psychoactive drugs and relevance in drug-induced reward. *Neuropharmacology* **63**, 224–234. https://doi.org/10.1016/j.neuropharm.2012.03.013 (2012).
49. Ashley, C. B. et al. AcoustilytixTM: A web-based automated ultrasonic vocalization scoring platform. *Brain Sci.* **11**, 864. https://doi.org/10.3390/brainsci11070864 (2021).
50. Johnson, A. M. et al. Semi-Automated Training of Rat Ultrasonic Vocalizations. *Front. Behav. Neurosci.* **16**, 826550. https://doi.org/10.3389/fnbeh.2022.826550 (2022).
51. Wang, H. et al. Ultrasonic vocalizations induced by sex and amphetamine in M2, M4, M5 muscarinic and D2 dopamine receptor knockout mice. *PLoS One*. **3**, e1893. https://doi.org/10.1371/journal.pone.0001893 (2008).
52. Simola, N., Ma, S. T. & Schallert, T. Influence of acute caffeine on 50-kHz ultrasonic vocalizations in male adult rats and relevance to caffeine-mediated psychopharmacological effects. *Int. J. Neuropsychopharmacol.* **13**, 123–132. https://doi.org/10.1017/S1461145709990113 (2010).
53. Burgdorf, J., Knutson, B., Panksepp, J. & Ikemoto, S. Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behav. Neurosci.* **115**, 940–944. https://doi.org/10.1037/0735-7044.115.4.940 (2001).
54. Thompson, B., Leonard, K. C. & Brudzynski, S. M. Amphetamine-induced 50 kHz calls from rat nucleus accumbens: A quantitative mapping study and acoustic analysis. *Behav. Brain Res.* **168**, 64–73. https://doi.org/10.1016/j.bbr.2005.10.012 (2006).
55. Ciucci, M. R. et al. Qualitative changes in ultrasonic vocalization in rats after unilateral dopamine depletion or haloperidol: A preliminary study. *Behav. Brain. Res.* **182**, 284–289. https://doi.org/10.1016/j.bbr.2007.02.020 (2007).
56. Costa, G. et al. Influence of dopamine transmission in the medial prefrontal cortex and dorsal striatum on the emission of 50-kHz ultrasonic vocalizations in rats treated with amphetamine: Effects on drug-stimulated and conditioned calls. *Prog Neuropsychopharmacol. Biol. Psychiatry*. **97**, 109797. https://doi.org/10.1016/j.pnpbp.2019.109797 (2020).
57. Krasko, M. N. et al. Rat models of vocal deficits in Parkinson's disease. *Brain Sci.* **11**, 925. https://doi.org/10.3390/brainsci11070925 (2021).
58. Simola, N. et al. Increased emissions of 50-kHz ultrasonic vocalizations in hemiparkinsonian rats repeatedly treated with dopaminomimetic drugs: A potential preclinical model for studying the affective properties of dopamine replacement therapy in Parkinson's disease. *Prog Neuropsychopharmacol. Biol. Psychiatry*. **108**, 110184. https://doi.org/10.1016/j.pnpbp.2020.110184 (2021).
59. Tassin, J. P. et al. Non-dopaminergic fibres may regulate dopamine-sensitive adenylate cyclase in the prefrontal cortex and nucleus accumbens **295**: 696–698. (1982). https://doi.org/10.1038/295696a0

60. Gerfen, C. R., Miyachi, S., Paletzki, R. & Brown, P. D1 Dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. *J. Neurosci.* **22**, 5042–5054. https://doi.org/10.1523/JNEUROSCI.22-12-05042.2002 (2002).
61. Simola, N., Morelli, M. & Carta, A. The 6-hydroxydopamine model of Parkinson's disease. *Neurotox. Res.* **11**, 151–167. https://doi.org/10.1007/BF03033565 (2007).
62. Grace, A. A. & Bunney, B. S. The control of firing pattern in nigral dopamine neurons: Burst firing. *J. Neurosci.* **4**, 2877–2890. https://doi.org/10.1523/jneurosci.04-11-02877.1984 (1984).
63. Willuhn, I. et al. Phasic dopamine release in the nucleus accumbens in response to pro-social 50 kHz ultrasonic vocalizations in rats. *J. Neurosci.* **34**, 10616–10623. https://doi.org/10.1523/JNEUROSCI.1060-14.2014 (2014).
64. Wright, J. M., Dobosiewicz, M. R. S. & Clarke, P. B. S. α- and β-Adrenergic receptors differentially modulate the emission of spontaneous and amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats. *Neuropsychopharmacology* **37**, 808–821. https://doi.org/10.1038/npp.2011.258 (2012).
65. Costa, G., Morelli, M. & Simola, N. Involvement of glutamate NMDA receptors in the acute, long-term, and conditioned effects of amphetamine on rat 50 kHz ultrasonic vocalizations. *Int. J. Neuropsychopharmacol.* **18**, pyv057. https://doi.org/10.1093/ijnp/pyv057 (2015).
66. Wöhr, M., Rippberger, H., Schwarting, R. K. W. & Van Gaalen, M. M. Critical involvement of 5-HT2C receptor function in amphetamine-induced 50-kHz ultrasonic vocalizations in rats. *Psychopharmacology* **232**, 1817–1829. https://doi.org/10.1007/s00213-014-3814-9 (2015).
67. Grant, L. M. et al. Noradrenergic receptor modulation influences the acoustic parameters of pro-social rat ultrasonic vocalizations. *Behav. Neurosci.* **132**, 269–283. https://doi.org/10.1037/bne0000258 (2018).
68. Burgdorf, J., Panksepp, J. & Moskal, J. R. Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. *Neurosci. Biobehav Rev.* **35**, 1831–1836. https://doi.org/10.1016/J.NEUBIOREV.2010.11.011 (2011).
69. Wöhr, M., Houx, B., Schwarting, R. K. W. & Spruijt, B. Effects of experience and context on 50-kHz vocalizations in rats. *Physiol. Behav.* **93**, 766–776. https://doi.org/10.1016/J.PHYSBEH.2007.11.031 (2008).
70. Simola, N., Frau, L., Plumitallo, A. & Morelli, M. Direct and long-lasting effects elicited by repeated drug administration on 50-kHz ultrasonic vocalizations are regulated differently: Implications for the study of the affective properties of drugs of abuse. *Int. J. Neuropsychopharmacol.* **17**, 429–441. https://doi.org/10.1017/S1461145713001235 (2014).
71. Serra, M., Costa, G., Onaivi, E. & Simola, N. Divergent acute and enduring changes in 50-kHz ultrasonic vocalizations in rats repeatedly treated with amphetamine and dopaminergic antagonists: New insights on the role of dopamine in calling behavior. *Int. J. Neuropsychopharmacol.* **27**, pyae001. https://doi.org/10.1093/ijnp/pyae001 (2024).
72. Taylor, J. O., Urbano, C. M. & Cooper, B. G. Differential patterns of constant frequency 50 and 22 kHz USV production are related to intensity of negative affective state. *Behav. Neurosci.* **131**, 115–126. https://doi.org/10.1037/bne0000184 (2017).
73. Yang, K. M. et al. Characterization of oromotor and limb motor dysfunction in the DJ1 -/- model of Parkinson disease. *Behav. Brain Res.* **339**, 47–56. https://doi.org/10.1016/J.BBR.2017.10.036 (2018).
74. Johnson, A., Grant, L., Schallert, T. & Ciucci, M. Changes in rat 50-kHz ultrasonic vocalizations during dopamine denervation and aging: Relevance to neurodegeneration. *Curr. Neuropharmacol.* **13**, 211–219. https://doi.org/10.2174/1570159x1302150525122416 (2015).
75. Shembel, A. C. et al. The role of ultrasonic vocalizations in rat laryngological investigations. *Physiol. Behav.* **294**, 114887. https://doi.org/10.1016/j.physbeh.2025.114887 (2025).
76. Simola, N. & Costa, G. Emission of categorized 50-kHz ultrasonic vocalizations in rats repeatedly treated with amphetamine or apomorphine: Possible relevance to drug-induced modifications in the emotional state. *Behav. Brain Res.* **347**, 88–98. https://doi.org/10.1016/j.bbr.2018.02.041 (2018).
77. Simola, N. Rat ultrasonic vocalizations and behavioral neuropharmacology: From the screening of drugs to the study of disease. *Curr. Neuropharmacol.* **13**, 164–179. https://doi.org/10.2174/1570159x13999150318113800 (2015).
78. Simola, N. & Granon, S. Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. *Neuropharmacology* **159**, 107420. https://doi.org/10.1016/j.neuropharm.2018.11.008 (2019).
79. Schwarting, R. K. W. Behavioral analysis in laboratory rats: Challenges and usefulness of 50-kHz ultrasonic vocalizations. *Neurosci. Biobehav Rev.* **152**, 105260. https://doi.org/10.1016/j.neubiorev.2023.105260 (2023).
80. Simola, N. & Morelli, M. Repeated amphetamine administration and long-term effects on 50-kHz ultrasonic vocalizations: Possible relevance to the motivational and dopamine-stimulating properties of the drug. *Eur. Neuropsychopharmacol.* **25**, 343–355. https://doi.org/10.1016/j.euroneuro.2015.01.010 (2015).
81. Williams, S. N. & Undieh, A. S. Dopamine-sensitive signaling mediators modulate psychostimulant-induced ultrasonic vocalization behavior in rats. *Behav. Brain Res.* **296**, 1–6. https://doi.org/10.1016/j.bbr.2015.08.008 (2016).
82. Serra, M., Marongiu, J., Simola, N. & Costa, G. Emission of 50-kHz ultrasonic vocalizations stimulated by antiparkinsonian dopaminomimetic drugs in hemiparkinsonian rats is associated with neuronal activation in subcortical regions that regulate the affective state. *Exp. Neurol.* **381**, 114939. https://doi.org/10.1016/j.expneurol.2024.114939 (2024).
83. Saini, T. & Mazumder, P. M. Current advancement in the preclinical models used for the assessment of diabetic neuropathy. *Naunyn Schmiedebergs Arch. Pharmacol.* **397**, 2727–2745. https://doi.org/10.1007/s00210-023-02802-0 (2024).
84. Jourdan, D., Ardid, D., Chapuy, E., Le Bars, D. & Eschalier, A. Effect of analgesics on audible and ultrasonic pain-induced vocalization in the rat. *Life Sci.* **63**, 1761–1768. https://doi.org/10.1016/s0024-3205(98)00450-0 (1998).
85. Oliveira, A. R. & Barros, H. M. Ultrasonic rat vocalizations during the formalin test: a measure of the affective dimension of pain? *Anesth. Analg.* **102**, 832–839. https://doi.org/10.1213/01.ane.0000196530.72813.d9 (2006).
86. Araya, E. I., Baggio, D. F., Koren, L. O., Schwarting, R. K. W. & Chichorro, J. G. Trigeminal neuropathic pain reduces 50-kHz ultrasonic vocalizations in rats, which are restored by analgesic drugs. *Eur. J. Pharmacol.* **922**, 174905. https://doi.org/10.1016/j.ejphar.2022.174905 (2022).
87. Saurin, Y. N. T. et al. Characterization of behavioral and emotional responses of male and female juvenile rats in the orofacial formalin test following acetaminophen treatment. Behav Brain Res. 2025;494:115748. (2025). https://doi.org/10.1016/j.bbr.2025.115748
88. Meyer zum Gottesberge, A. M. et al. Zucker diabetic fatty rats, a model for type 2 diabetes, develop an inner ear dysfunction that can be attenuated by losartan treatment. *Cell. Tissue Res.* **362**, 307–315. https://doi.org/10.1007/s00441-015-2215-7 (2015).
89. Lee, J. H., Ji, S. H., Jung, J. Y., Lee, M. Y. & Lee, C. K. High blood glucose levels affect auditory brainstem responses after acoustic overexposure in rats. *Audiol. Neurootol.* **26**, 257–264. https://doi.org/10.1159/000511448 (2021).
90. Stenzel, M. et al. Exploring the cognitive effects of hearing loss in adult rats: Implications for visuospatial attention, social behavior, and prefrontal neural activity. *Neuroscience* **564**, 97–109. https://doi.org/10.1016/j.neuroscience.2024.11.010 (2025).

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Author contributions

J.R., Conceptualization, Investigation, Writing - Original Draft, Methodology; J.B., Investigation, Methodology; J.M., Data curation, Formal analysis; G.C., Data curation, Formal analysis; M.G.M., Conceptualization, Funding acquisition, Resources, Writing - Original Draft; Writing - Review & Editing; N.S., Conceptualization, Data curation, Formal analysis; Writing - Original Draft; Writing - Review & Editing.

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Declarations

Competing interests

The authors declare no competing interests.

Authorship statement

This article presents a part of J.R.' s posthumous results. J.R. fulfils all the authorship criteria according to the standard interpretation of the Vancouver rules set by The International Committee of Medical Journal Editors (ICMJE) (Helgesson et al., 2019 J Med Ethics, https://doi.org/10.1136/medethics-2018-105304). This version of the article was approved by the Officer promoting the Responsible Conduct of Research at the Université du Québec in Trois-Rivières. All the co-authors agree to include J.R. as the first author of this article.

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