



HAL
open science

Courtship vocalizations: A potential biomarker of adult exposure to endocrine disrupting compounds?

Sakina Mhaouty-Kodja

► **To cite this version:**

Sakina Mhaouty-Kodja. Courtship vocalizations: A potential biomarker of adult exposure to endocrine disrupting compounds?. *Molecular and Cellular Endocrinology*, 2020, 501, pp.110664. 10.1016/j.mce.2019.110664 . hal-02400573

HAL Id: hal-02400573

<https://hal.science/hal-02400573>

Submitted on 23 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title

Courtship vocalizations: a potential biomarker of adult exposure to endocrine disrupting compounds?

Running title

Courtship vocalizations in rodents

Author

Sakina Mhaouty-Kodja

Affiliations

Sorbonne Université, CNRS, INSERM, Neuroscience Paris Seine – Institut de Biologie Paris-Seine, 75005 Paris, France.

Corresponding author

Sakina Mhaouty-Kodja

Sorbonne Université, CNRS UMR 8246, INSERM U1130

7 quai St Bernard, Bât A 3ème étage 75005, Paris, France.

Tel: +331 44 27 91 38

sakina.mhaouty-kodja@upmc.fr

Key words

Ultrasonic vocalizations; Courtship; Behavior; Sex steroids; Endocrine disruption; Rodents

Abstract

In rodents, male courtship is stimulated by pheromones emitted by the sexually receptive female. In response, the male produces ultrasonic vocalizations, which appear to play a role in female attraction and facilitate copulation. The present review summarizes the main findings on courtship vocalizations and their tight regulation by sex steroid hormones. It describes studies that address the effects of exposure to endocrine disrupting compounds (EDC) on ultrasound production, as changes in hormone levels or their signaling pathways may interfere with the emission of ultrasonic vocalizations. It also discusses the potential use of this behavior as a noninvasive biomarker of adult exposure to EDC.

1. Introduction

Rodents produce ultrasonic vocalizations (USVs) in a wide variety of social contexts, such as mother-pup retrieval and interactions between juveniles or between adult individuals of either the same or opposite sex. Rodent USVs have been recognized for more than a century (Sidebotham, 1877), recorded for the first time in the fifties (Zippelius & Schleidt, 1956), and studied in the context of sexual interaction since the 70's (Nyby et al. 1977; Nyby, 1983; Sales, 1972; Whitney et al. 1973; Whitney et al. 1974). Nevertheless, they have only recently attracted considerable academic interest since the discovery of their song-like structure (Holy & Guo, 2005).

Here, I summarize the main findings obtained on USVs emitted by males during sexual interaction, their regulation by sex steroid hormones, and their potential alteration by exposure to endocrine disrupting compounds (EDCs). EDCs are defined by the WHO and the Endocrine Society as exogenous chemical substances or mixtures of substances that alter functions of the endocrine system. EDC exposure has been reported to alter several reproductive functions (Who, 2012), including mating behavior (reviewed in Gore et al., 2018; Mhaouty-Kodja et al., 2018). To date, very few studies have addressed the effects of EDCs on the emission of courtship vocalizations.

2. Male ultrasonic vocalizations during courtship

Courtship behavior is essential for sexual reproduction and constitutes a chain of behavioral responses that lead to mating. Vocalizations have been extensively studied in songbirds in this context and have been shown to play a key role in female attraction and the success of sexual reproduction. Only recently has the emission of USVs during courtship gained new interest in mice, as mentioned above (Chabout et al., 2015; Egnor & Seagraves, 2016; Holy & Guo, 2005).

In rodents, the courting male displays a chemo-investigating behavior towards the receptive female. In response to females or their odors, males produce USVs (Bean, 1982; Dizinno & Whitney, 1977; Nyby et al., 1977), which display the characteristics of songs (Holy & Guo, 2005). The vocalization

rate drops dramatically following the first mounting events (Hanson & Hurley, 2012) and the male engages in the copulatory phase by exhibiting thrusting and intromitting behaviors before reaching ejaculation.

The emission of USVs under mating conditions are assessed by performing recordings in the presence of a sexually receptive female (Figure 1A). Under such conditions, males emit most of the produced USVs, whereas females vocalize in other social contexts towards female congeners or their young (D'Amato et al., 2001; Hanson & Hurley, 2012; Maggio & Whitney, 1985; Moles et al., 2007; Wang et al., 2008; Whitney et al., 1973). In particular, devocalization of male mice reduces USV production to a low level, which is comparable to the condition in which both male and female partners are devocalized (White et al., 1998). A recent study using a new microphone array system to localize vocalizations from interacting mice showed approximately 82% of vocal signal counts to be attributable to males (Neunuebel et al., 2015). These data were more recently confirmed by reports showing that females vocalize significantly less often than males during dyadic interactions, with females accounting for approximately one sixth of all emitted signals (Warren et al., 2018).

The emission of courtship USVs conveys information about the male's motivational state and plays a role in attracting and maintaining the sexual interest of the female partner (Floody et al., 1979, Pomerantz et al., 1983; Thomas et al., 1981). For example, females spend more time near vocalizing than surgically devocalized males (Pomerantz et al., 1983) and playback of male USVs elicited approach behavior of females in a place preference paradigm (Hammerschmidt et al., 2009). USVs have also been suggested to provide information about male quality, as in songbirds, since USVs are associated with dominance (Nyby et al., 1976) and regulated by androgens, as detailed in section 5.

3. Features of courtship USVs in rodents

Male rodents mainly vocalize at a frequency of 35 to 110 kHz. In mice, USVs consist of several syllable types that are organized into phrases (Holy & Guo, 2005). The characteristics of USVs in terms of syllable composition, frequency of emission, duration, and frequency of the waveform category are different between mouse strains (Kikusui et al., 2011; Sugimoto et al., 2011). In male mice with a C57BL/6 background, nine major syllables that vary in duration and complexity have been identified and categorized as short, flat, upward, downward, modulated, complex, mixed, one jump, and with frequency jumps and grouped into three broad categories (Figure 1B) (Capela et al., 2018; Dombret et al., 2017; Ey et al., 2013). The number and duration of syllables emitted during sexual interaction are quantifiable. However, the meaning of each syllable is not clearly known and the most appealing types of syllables emitted by males towards female have not been identified thus far. Nevertheless, Chabout *et al.* (2015) proposed that male mice emit simple syllables for direct courtship when the female is present and more complex “calling songs”, intended to attract the females, when males can smell but not see them. In another study, Matsumoto and Okanoya (2016) observed that male mice emit short syllables during the first minutes of interaction and longer and more complex syllables with harmonic sounds during mounting. Finally, it was observed that males emit more “up” and “modulated” (U-shaped) syllables and less “flat” and “down” syllables when females were removed from the cage (Hanson & Hurley, 2012).

The emission of USVs appears to be an innate character, as congenital deaf mice or animals rendered deaf at postnatal day (PND) 2 are able to emit ultrasonic vocalizations with characteristics identical to those observed in their hearing controls (Hammerschmidt et al., 2015; Mahrt et al., 2013). Furthermore, a cross-fostering experiment carried out between birth and PND21 showed that mice placed with foster parents with a genetic background different from theirs emitted vocalizations with acoustics similar to those of their genetic parents (Kikusui et al., 2011). Nevertheless, some studies have suggested that learning may occur under certain conditions. In a study of Arriaga *et al.* (2012), adult male mice were able to change some spectral features of their

songs under conditions of social competition, in which a male was placed in the presence of a receptive female and a male of a different strain. The authors concluded that male mice have some ability to modify their vocalizations, albeit limited, with at least some neuroanatomical features thought to be unique to humans and song-learning birds.

4. Neural systems that control the emission of USVs

The neural circuitry that processes the expression of sexual behavior in rodents is activated by pheromone cues emitted by a sexually receptive female. Signals are detected by the olfactory system and transmitted to the medial amygdala, bed nucleus of the stria terminalis, and the medial preoptic area, where signals are processed into behavioral responses, such as partner preference, urine marking (Figure 2). Courtship USVs are also produced in response to females or their odors (Bean, 1982; Dizinno & Whitney, 1977; Nyby et al., 1977), yet the neural structure underlying the motivation to produce USVs still needs to be documented in rodents, in particular the precise role of the medial preoptic area. Lesion of this hypothalamic area in adult male mice reduced mounting behavior but did not affect the ultrasonic vocalizations emitted in the presence of a female (Bean et al., 1981). However, blockade of protein synthesis by anisomycin into the rat medial preoptic area significantly decreased both the expression of male sexual behavior and ultrasonic vocalizations (McGinnis & Kahn, 1997). More recently, Gao et al. (2019) reported that optogenetic stimulation of the medial preoptic area, in particular neurons expressing the vesicular gamma-aminobutyric acid transporter-positive (Vgat), elicited the emission of USVs by males in the absence of any female stimulus. These USVs were acoustically similar to those emitted spontaneously by adult males in the presence of a female. Interestingly, ablation of these Vgat neurons did not reduce USV levels but altered spectral features of courtship USVs.

A study by Arriaga *et al.* (2012) showed that the mouse song system responsible for USV production by males includes two pathways that converge at the nucleus ambiguus, which innervates the larynx muscles. One pathway originates from the periaqueductal grey and the other

from the primary motor cortex. The periaqueductal grey area could represent a potential key region connecting the motor system to the neural circuitry underlying sexual behavior. Indeed, it receives projections from the medial hypothalamus and preoptic area in rats and mice (Kohl et al., 2018; Shipley & Ennis, 1991) and was shown to induce mating calls when electrically stimulated at the level of the caudal subregion in the male guinea pig (Kyhou & Gemba, 1998). Finally, silencing a subset of neurons in the periaqueductal grey selectively abolished courtship vocalizations and impaired their ability to attract female mice in a social choice assay (Tschida et al., 2018). By contrast, optogenetic activation of these neurons elevated USV production, even in the absence of female cues.

5. Hormonal regulation of courtship USVs and underlying signaling pathways

Several rodent studies have reported that the USVs emitted by males in response to females or their urine-soiled bedding, are modulated by testicular testosterone. Castration of adult males was shown to induce a decline in ultrasound production, which was restored by testosterone treatment for the 70-kHz vocalizations in mice (Bean et al., 1986; Dizinno & Whitney, 1977; Nunez et al., 1978; Nunez & Tan, 1984), 35-kHz vocalizations in deer mice (Pomerantz et al., 1983) and hamsters (Floody et al., 1979), and 50-kHz calls in rats (Matochik & Barfield, 1991). In these studies, castration did not completely abolish ultrasound production, but few vocalizations were emitted in the presence of females, suggesting a role of testosterone in the motivation to vocalize rather than a deficit in an effector system (Nunez et al., 1978). The testosterone metabolites estradiol and 5 α -dihydrotestosterone restore USV production at different levels, depending on the doses used, but are as effective as testosterone when treatments combine both hormones (Bean et al., 1986; Matochik et al., 1991; Nunez et al., 1978; Nunez & Tan, 1984; Pomerantz et al., 1983). Another argument in favor of the involvement of both androgen and estrogen signaling pathways in the hormonal regulation of courtship USVs comes from the use of systemic administration of androgen receptor (AR) or estrogen receptor (ER) antagonists, which block the 50-kHz ultrasonic vocalizations of rats,

either when used alone or together (Vagell et al., 1998). In addition to the long-term effects of sex steroids, recent studies have shown that testosterone and estradiol can also rapidly alter the duration and frequency of USVs in hamsters (Fernández-Vargas, 2017). Overall, these data support the idea that USVs are dynamic signals that can rapidly change in response to both internal and external environments.

At the neural level, several studies designate the medial preoptic nucleus as the key structure that integrates the hormonal signals necessary to induce the motivation to produce USVs during courtship. Ultrasonic mating vocalizations were completely restored in castrated male mice receiving implants of testosterone into the medial preoptic area, but not the corticomedullar amygdala, septum, anterior and ventromedial hypothalamus, or ventral tegmental area (Matochik et al., 1994; Nyby, 1992; Sipos & Nyby, 1996; Sipos & Nyby, 1998). A study in rats showed similar results, with implants in the ventromedial hypothalamus restoring partner preference but not 50-kHz vocalizations (Harding & McGinnis, 2003). Estradiol benzoate implanted into the preoptic area also activated ultrasonic vocalizations in male mice and gerbils (Holman et al., 1991; Nyby, 1992).

The involvement of neural AR was first studied by implanting the anti-androgen hydroxyflutamide in the medial preoptic area or the ventromedial nucleus of castrated males supplemented with testosterone (Harding & McGinnis, 2004). It was found that AR blockade in the ventromedial nucleus was more effective in inhibiting the restoration of 50-kHz vocalizations. More recently, the neural AR role in testosterone-induced regulation of USVs was investigated using a transgenic mouse model lacking the *AR* in the nervous system (Raskin et al., 2009). Under equivalent testosterone levels, naïve mutant males displayed a smaller total number and shorter duration of USVs in the presence of receptive females than their control littermates (Dombret et al., 2017). They also exhibited a smaller number of emitted syllables (-57% to -70% *versus* controls) for seven of the nine syllables (short, flat, upward, modulated, complex, one-jump, and frequency jump). The duration was also reduced for short, modulated, complex, one-jump, and frequency-jump syllables.

Our studies have also shown that all control males were able to vocalize in the presence of sexually receptive females after a first sexual experience, whereas the percentage remained unchanged for mutants (Figure 3A). Two-way ANOVA showed an effect of genotype and experience on the total number and duration of USVs (Figure 3B-C). Despite an increase in both parameters in the mutant males after a first sexual experience, they were still lower than those for their control littermates (Figure 3C). These data confirm previous studies concerning the effect of sexual experience on the emission of courtship USVs (Nunez & Tan, 1984) and suggest that the neural AR mediates, at least in part, the testosterone-induced regulation of ultrasonic vocalizations. Current studies are investigating the participation of neural estrogen receptors.

6. Effect of exposure to EDCs

The hormone-dependent regulation of courtship USVs suggests that this behavior may be sensitive to exposure to EDCs, as for other components of sexual behavior (Mhaouty-Kodja et al., 2018). Exposure to EDCs can interfere with the emission of courtship vocalizations through changes in sex steroid levels or their signaling pathways in the neural circuitry underlying sexual behavior or motor system controlling ultrasonic vocalizations (Figure 2). Five studies have been conducted on the effects of exposure to environmental EDCs on USVs emitted in the context of sexual interaction (Table 1). Three focused on developmental exposure to octylphenol (Pocock et al., 2002), polychlorinated biphenyls (PCBs), and vinclozolin (Bell et al., 2016; Krishnan et al., 2018), whereas the other two addressed the effects of adult exposure to di-(2-ethylexyl)phthalate (DEHP) and nonylphenol (NP) (Capela et al., 2018; Dombret et al., 2017).

6.1. Developmental exposure

Octylphenol and NP result from the degradation of alkylphenols, which are used as nonionic surfactants in a wide variety of industrial, agricultural, and domestic applications, and exhibit estrogenic activity (Kwack et al., 2002; Laws et al., 2000; Yadetie et al., 1999). Vinclozolin, a

fungicide used on fruits and vegetables, and DEHP, the most abundant phthalate used in the manufacture and processing of plastic products, are known to exhibit anti-androgenic activities (Gray et al., 1994; Wolf et al., 1999). PCBs are persistent chemicals, banned in the 70 and 80's, previously used as fire preventives in electrical equipment. They have been reported to alter several physiological systems, including the thyroid and reproduction (Gray et al., 1993).

Prenatal and postnatal exposure to octylphenol at high doses (100-250 mg/kg/day) or PCB (1 mg/kg) had no effects on USVs emitted by adult males of the F1 generation (Bell et al., 2016; Pocock et al., 2002). Exposure to PCB or vinclozolin (1 mg/kg each) lowered the rate and frequency of USVs emitted by adult males of the F2 generation (Krishnan et al., 2018). These changes were associated with lower levels of estradiol and testosterone in the PCB- and vinclozolin-exposed groups. Such long-term induced modifications in hormonal levels could underlie, at least partially, the observed behavioral changes.

Further studies are needed to clarify the effects of developmental exposure to EDCs on courtship USVs, in particular during the perinatal and prepubertal periods, when testosterone and its metabolites are required for the sexual differentiation of brain areas involved in sexual behavior and USVs. In this context, special attention should be given to EDCs with anti-androgenic, estrogenic, or anti-estrogenic activity.

6.2. Adult exposure

The two remaining studies addressed the effects of adult exposure to low doses (0.5, 5 and 50 µg/kg/day) of DEHP and NP in male mice (Capela et al., 2018; Dombret et al., 2017). We used the same protocol of chronic exposure and showed that the effects of adult exposure to each of these compounds on USVs and sexual behavior present similarities and differences.

Exposure to DEHP lowered the total number and duration of USVs. A detailed analysis of the syllables showed changes in the frequency of eight syllables and the duration of five at a dose of 50

$\mu\text{g}/\text{kg}/\text{day}$. Lower doses of 0.5 and 5 $\mu\text{g}/\text{kg}/\text{day}$ affected the frequency and duration of short and flat syllables and slightly altered the ratio of syllable categories. These effects on courtship vocalizations were associated with diminished sexual interest of female partners towards males exposed to 5 or 50 $\mu\text{g}/\text{kg}/\text{d}$. Males of these two-exposed groups took longer to initiate mating, despite normal olfactory preference (Dombret et al., 2017). Further analyses showed that such behavioral changes were not due to modifications in hormonal levels or the integrity of the hypothalamus-pituitary axis but rather to down-regulation of the AR in the neural circuitry underlying the expression of sexual behavior (medial amygdala, bed nucleus of stria terminalis, and medial preoptic area).

Exposure of adult male mice to NP induced most of the effects at a dose of 5 $\mu\text{g}/\text{kg}/\text{d}$ (Capela et al., 2018). In contrast to DEHP, NP exposure resulted in an increase in the total number and duration of USVs. In particular, changes concerned the total number and duration of the frequency-jump category and the total and mean duration of syllables of all categories. The latency to initiate mating was unchanged but the duration of mating increased, with a significantly higher number of mounts, intromissions, and thrusts exhibited by males exposed to NP at 5 $\mu\text{g}/\text{kg}/\text{d}$ than those of the other groups. These modifications are similar to those induced by estrogens on USVs (Bean et al., 1986; Nunez et al., 1978; Nyby et al., 1992; Pomerantz et al., 1983) and copulatory behavior (Retana-Marquez et al., 2016). As for DEHP, adult exposure to NP did not trigger any changes in testosterone levels or the hypothalamic-pituitary-gonadal axis but altered the number of AR- and ER α -immunoreactive cells in the medial amygdala and bed nucleus of the stria terminalis and AR protein levels in the medial preoptic area (Capela et al., 2018).

Among the various behaviors analyzed in these two studies, USVs appeared sufficient to predict the anti-androgenic effect of DEHP and estrogenic-like effect of NP. Such activities were confirmed by the combination of several approaches and involved, at least in part, modifications in the levels of neural AR and ER α .

7. Conclusion

As for other components of sexual behavior, courtship USVs are tightly regulated by testosterone and its metabolites. Alterations in hormonal levels or their signaling pathways could, therefore, interfere with USV emission. The data obtained following chronic exposure of male mice during adulthood indicate that the recording of ultrasounds may suggest the anti-androgenic or estrogenic activities of studied molecules. The high sensitivity of adult neural systems involved in the motivation to vocalize and/or produce USVs to DEHP and NP exposure is shown by the low doses at which the effects were induced. Whether the effects of adult exposure to DEHP and NP on USV emission can be reversed following the discontinuation of exposure is yet to be studied. Also, further studies addressing adult exposure are required to determine whether the observations made in the DEHP and NP studies extend to other molecules that exhibit anti-androgenic or estrogenic activities. The potential use of courtship USVs to directly predict the effects and activity of EDCs provides interesting perspectives in the context of characterizing and developing noninvasive *in vivo* biomarkers of endocrine disruption not only in rodents but in other species as well. Indeed, fish, amphibians, birds, and mammals have in common the use of vocalizations during courtship, which are regulated by testosterone in all (Bass & Remage-Healey, 2008). Thus, the study of vocalizations in diverse species may reveal their vulnerability to exposure to environmental EDCs.

Acknowledgments

This work was supported by the grant (Project n° 2012-2-077) from the “Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail” (Anses).

References

- Arriaga G, Zhou EP, Jarvis ED. Of mice, birds, and men: the mouse ultrasonic song system has some features similar to humans and song-learning birds. *PLoS One* 2012, 7: e46610.
- Bass AH, Remage-Healey L. Central pattern generators for social vocalization: Androgen-dependent neurophysiological mechanisms. *Horm Behav.* 2008, 53: 659-672.
- Bean NJ, Nyby J, Kerchner M, Dahinden Z. Hormonal regulation of chemosignal-stimulated precopulatory behaviors in male housemice (*Mus musculus*). *Horm Behav.* 1986, 20: 390-404.
- Bean NJ. Olfactory and vomeronasal mediation of ultrasonic vocalizations in male mice. *Physiol Behav.* 1982, 28: 31-37.
- Bean NY, Nunez AA, Conner R. Effects of medial preoptic lesions on male mouse ultrasonic vocalizations and copulatory behavior. *Brain Res Bull.* 1981, 6(2):109-112.
- Bell MR, Thompson LM, Rodriguez K, Gore AC. Two-hit exposure to polychlorinated biphenyls at gestational and juvenile life stages: 1. Sexually dimorphic effects on social and anxiety-like behaviors. *Horm Behav.* 2016, 78:168-177.
- Capela D, Dombret C, Poissenot K, Poignant M, Malbert-Colas A, Franceschini I, Keller M, Mhaouty-Kodja S. Adult male mice exposure to nonylphenol alters courtship vocalizations and mating. *Sci Rep.* 2018, 8: 2988.
- Chabout J, Sarkar A, Dunson DB, Jarvis ED. Male mice song syntax depends on social contexts and influences female preferences. *Front Behav Neurosci.* 2015, 9: 76.
- D'Amato FR, Moles A. Ultrasonic vocalizations as an index of social memory in female mice. *Behav Neurosci.* 2001, 115:834-840.
- Dizinno G, Whitney G. Androgen influence on male mouse ultrasounds during courtship. *Horm Behav.* 1977, 8: 188-192.
- Dombret C, Capela D, Poissenot K, Parmentier C, Bergsten E, Pionneau C, Chardonnet S, Grange-Messent V, Keller K, Franceschini I, Mhaouty-Kodja S. Neural mechanisms underlying

- disruption of male courtship behavior by adult exposure to di-(2-ethylhexyl)phthalate in mice. *Environ Health Perspect.* 2017, 125: 097001.
- Egnor SR, Seagraves KM. The contribution of ultrasonic vocalizations to mouse courtship. *Curr Opin Neurobiol.* 2016, 38: 1-5.
- Ey E, Torquet N, Le Sourd AM, Leblond CS, Boeckers TM, Faure P, Bourgeron T. The Autism ProSAP1/Shank2 mouse model displays quantitative and structural abnormalities in ultrasonic vocalisations. *Behav Brain Res.* 2013, 256: 677-689.
- Fernández-Vargas M. Rapid effects of estrogens and androgens on temporal and spectral features in ultrasonic vocalizations. *Horm Behav.* 2017, 94: 69-83.
- Floody OR, Walsh C, Flanagan MT. Testosterone stimulates ultrasound production by male hamsters. *Horm Behav.* 1979, 12: 164-171.
- Gao SC, Wei YC, Wang SR, Xu XH. Medial preoptic area modulates courtship ultrasonic vocalization in adult male mice. *Neurosci Bull.* 2019, 35(4): 697-708.
- Gore AC, Holley AM, Crews D. Mate choice, sexual selection, and endocrine-disrupting chemicals. *Horm Behav.* 2018, 101: 3-12.
- Gray LE Jr, Ostby J, Marshall R, Andrews J. Reproductive and thyroid effects of low-level polychlorinated biphenyl (Aroclor 1254) exposure. *Fundam Appl Toxicol.* 1993, 20: 288-294.
- Gray LE Jr, Ostby JS, Kelce WR. Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. *Toxicol Appl Pharmacol.* 1994, 129: 46-52.
- Hammerschmidt K, Radyushkin K, Ehrenreich H, Fischer J. Female mice respond to male ultrasonic 'songs' with approach behaviour. *Biol Lett.* 2009, 5: 589-592.
- Hammerschmidt K, Schreiweis C, Minge C, Pääbo S, Fischer J, Enard W. A humanized version of Foxp2 does not affect ultrasonic vocalization in adult mice. *Genes Brain Behav.* 2015, 14: 583-90.

- Hanson JL, Hurley LM. Female presence and estrous state influence mouse ultrasonic courtship vocalizations. *PLoS ONE* 2012, 7: e40782.
- Harding SM, McGinnis MY. Androgen receptor blockade in the MPOA or VMN: effects on male sociosexual behaviors. *Physiol Behav.* 2004, 81: 671-680.
- Harding SM, McGinnis MY. Effects of testosterone in the VMN on copulation, partner preference, and vocalizations in male rats. *Horm Behav.* 2003, 43: 327-335.
- Holman SD, Hutchison RE, Hutchison JB. Microimplants of estradiol in the sexually dimorphic area of the hypothalamus activate ultrasonic vocal behavior in male Mongolian gerbils. *Horm Behav.* 1991, 25: 531-548.
- Holy TE, Guo Z. Ultrasonic songs of male mice. *PLoS Biol.* 2005, 3: e386.
- Kikusui T, Nakanishi K, Nakagawa R, Nagasawa M, Mogi K, Okanoya K. Cross fostering experiments suggest that mice songs are innate. *PLoS One* 2011, 6: e17721.
- Kohl J, Babayan BM, Rubinstein ND, Autry AE, Marin-Rodriguez B, Kapoor V, Miyamishi K, Zweifel LS, Luo L, Uchida N, Dulac C. Functional circuit architecture underlying parental behaviour. *Nature* 2018, 556: 326-331.
- Krishnan K, Mittal N, Thompson LM, Rodriguez-Santiago M, Duvauchelle CL, Crews D, Gore AC. Effects of the endocrine-disrupting chemicals, vinclozolin and polychlorinated biphenyls, on physiological and sociosexual phenotypes in F2 generation Sprague-Dawley rats. *Environ Health Perspect.* 2018, 126: 97005.
- Kwack SJ, Kwon O, Kim HS, Kim SS, Kim SH, Sohn KH, Lee RD, Park CH, Jeung EB, An B-S. Comparative evaluation of alkylphenolic compounds on estrogenic activity in vitro and in vivo. *J Toxicol Environ Health Part A.* 2002, 65: 419-431.
- Kyuhou S, Gemba H. Two vocalization-related subregions in the midbrain periaqueductal gray of the guinea pig. *Neuroreport* 1998, 9: 1607-1610.
- Laws SC, Carey SA, Ferrell JM, Bodman GJ, Cooper RL. Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. *Toxicol Sci.* 2000, 54: 154-167.

Maggio JC, Whitney G. Ultrasonic vocalizing by adult female mice (*Mus musculus*). *J Comp Psychol.* 1985, 99: 420-436.

Mahrt EJ, Perkel DJ, Tong L, Rubel EW, Portfors CV. Engineered deafness reveals that mouse courtship vocalizations do not require auditory experience. *J Neurosci.* 2013, 33: 5573-83.

Matochik JA, Barfield RJ. Hormonal control of precopulatory sebaceous scent marking and ultrasonic mating vocalizations in male rats. *Horm Behav.* 1991, 25: 445-460.

Matochik JA, Sipos ML, Nyby JG, Barfield RJ. Intracranial androgenic activation of male-typical behaviors in house mice: motivation versus performance. *Behav Brain Res.* 1994, 60: 141-149.

Matsumoto YK, Okanoya K. Phase-specific vocalizations of male mice at the initial encounter during the courtship sequence. *PLoS One* 2016, 11: e0147102.

McGinnis MY, Kahn DF. Inhibition of male sexual behavior by intracranial implants of the protein synthesis inhibitor anisomycin into the medial preoptic area of the rat. *Horm Behav.* 1997, 31(1): 15-23.

Mhaouty-Kodja S, Naulé L, Capela D. Sexual behavior: From hormonal regulation to endocrine disruption. *Neuroendocrinology* 2018, 107: 400-416.

Moles A, Costantini F, Garbugino L, Zanettini C, D'Amato FR. Ultrasonic vocalizations emitted during dyadic interactions in female mice: a possible index of sociability? *Behav Brain Res.* 2007, 182: 223-230.

Neunuebel JP, Taylor AL, Arthur BJ, Egnor SER. Female mice ultrasonically interact with males during courtship displays. *Elife* 2015, 4: e06203.

Nunez AA, Nyby J, Whitney G. The effects of testosterone, estradiol, and dihydrotestosterone on male mouse (*Mus musculus*) ultrasonic vocalizations. *Horm Behav.* 1978, 11:264-272.

Nunez AA, Tan DT. Courtship ultrasonic vocalizations in male Swiss-Webster mice: effects of hormones and sexual experience. *Physiol Behav.* 1984, 32: 717-721.

Nyby J, Dizinno GA, Whitney G. Social status and ultrasonic vocalizations of male mice. *Behav Biol.* 1976, 18:285-289.

- Nyby J, Matochik JA, Barfield RJ. Intracranial androgenic and estrogenic stimulation of male-typical behaviors in house mice (*Mus domesticus*). *Horm Behav.* 1992, 26: 24-45.
- Nyby J, Wysocki CJ, Whitney G, Dizinno G. Pheromonal regulation of male mouse ultrasonic courtship (*Mus musculus*). *Anim Behav.* 1977, 25: 333-341.
- Nyby J. Ultrasonic vocalizations during sex behavior of male house mice (*Mus musculus*): a description. *Behav Neural Biol.* 1983, 39: 128-134.
- Pocock VJ, Sales GD, Wilson CA, Milligan SR. Effects of perinatal octylphenol on ultrasound vocalization, behavior and reproductive physiology in rats. *Physiol Behav.* 2002, 76: 645-653.
- Pomerantz SM, Fox E, Clemens LG. Gonadal hormone activation of male courtship ultrasonic vocalizations and male copulatory behavior in castrated male deer mice (*Peromyscus maniculatus bairdi*). *Behav Neurosci.* 1983, 97: 462-469.
- Raskin K, de Gendt K, Duittoz A, Liere P, Verhoeven G, Tronche F, Mhaouty-Kodja S. Conditional inactivation of androgen receptor gene in the nervous system: effects on male behavioral and neuroendocrine responses. *J Neurosci.* 2009, 29: 4461-4470.
- Raskin K, Marie-Luce C, Picot M, Bernard V, Mailly P, Hardin-Pouzet H, Tronche F, Mhaouty-Kodja S. Characterization of the spinal nucleus of the bulbocavernosus neuromuscular system in male mice lacking androgen receptor in the nervous system. *Endocrinology* 2012, 53: 3376-3385.
- Retana-Márquez S, Juárez-Rojas L, Hernández A, Romero C, López G, Miranda L, Guerrero-Aguilera A, Solano F, Hernández E, Chemineau P, Keller M, Delgadillo JA. Comparison of the effects of mesquite pod and *Leucaena* extracts with phytoestrogens on the reproductive physiology and sexual behavior in the male rat. *Physiol Behav.* 2016, 164: 1-10.
- Sales GD. Ultrasound and mating behavior in rodents with some observations on other behavioral situations. *J Zool.* 1972, 168: 149-164.
- Shipley MT, Ennis M. The Midbrain periaqueductal gray matter. In: Depaulis A and R. Bandler eds. New York: Plenum Press, 1991: 417-448.

- Sidebotham J. Singing mice. *Nature* 1877; 17: 29.
- Sipos ML, Nyby JG. Concurrent androgenic stimulation of the ventral tegmental area and medial preoptic area: synergistic effects on male-typical reproductive behaviors in house mice. *Brain Res.* 1996, 729: 29-44.
- Sipos ML, Nyby JG. Intracranial androgenic activation of male-typical behaviours in house mice: concurrent stimulation of the medial preoptic area and medial nucleus of the amygdala. *J Neuroendocrinol.* 1998, 10: 577-586.
- Sugimoto H, Okabe S, Kato M, Koshida N, Shiroishi T, Mogi K, Kikusui T, Koide T. A role for strain differences in waveforms of ultrasonic vocalizations during male-female interaction. *PLoS One* 2011, 6(7):e22093.
- Thomas DA, Talalas L, Barfield RJ. Effect of devocalization of the male on mating behavior in rats. *J Comp Physiol Psychol.* 1981, 95: 630-637.
- Tschida K, Michael V, Han BX, Zhao S, Sakurai K, Mooney R, Wang F. Identification of midbrain neurons essential for vocal communication. *BioRxiv* 2018.
- Vagell ME, McGinnis MY. The role of gonadal steroid receptor activation in the restoration of sociosexual behavior in adult male rats. *Horm Behav.* 1998, 33: 163-179.
- Wang H, Liang S, Burgdorf J, Wess J, Yeomans J. Ultrasonic vocalizations induced by sex and amphetamine in M2, M4, M5 muscarinic and D2 dopamine receptor knockout mice. *PLoS One* 2008, 3: e1893.
- Warren MR, Spurrier MS, Roth ED, Neunuebel JP. Sex differences in vocal communication of freely interacting adult mice depend upon behavioral context. *PLoS One* 2018, 13(9):e0204527.
- White NR, Prasad M, Barfield RJ, Nyby JG. 40- and 70-kHz vocalizations of mice (*Mus musculus*) during copulation. *Physiol Behav.* 1998, 63: 467-473.
- Whitney G, Alpern M, Dizinno G, Horowitz G. Female odors evoke ultrasounds from male mice. *Anim Learn Behav.* 1974, 2: 13-18.

- Whitney G, Coble JR, Stockton MD, Tilson EF. Ultrasonic emissions: do they facilitate courtship of mice. *J Comp Physiol Psychol*. 1973, 84: 445-452.
- WHO (World Health Organization). 2012. State of the science of endocrine disrupting chemicals. Available: <http://www.who.int/ceh/publications/endocrine/en/> [accessed 24 October 2016].
- Wolf C Jr, Lambright C, Mann P, Price M, Cooper RL, Ostby J, Gray LE Jr. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicol Ind Health*. 1999, 15: 94-118.
- Yadete F, Arukwe A, Goksøyr A, Male R. Induction of hepatic estrogen receptor in juvenile Atlantic salmon in vivo by the environmental estrogen, 4-nonylphenol. *Sci Total Environ*. 1999, 233: 201-210.
- Zippelius HM, Schleidt WM. Ultraschall-Laute bei jungen Mäusen. *Naturwissenschaften* 1956, 43: 502.

Figure legends

Figure 1. A) Recording of courtship ultrasonic vocalizations. Males are tested for 4 to 5 min in their home cage under red-light illumination, 2 h after lights off. The recording of vocalizations starts after the introduction of a sexually receptive female using a microphone connected to an ultrasound recording interface plugged into a computer equipped with the recording software. The total number and duration of USVs are quantified. The number and duration of each syllable, identified as in Figure 1, can also be analyzed. **B)** Illustration of ultrasonic vocalizations in mice. Nine syllable types of ultrasonic vocalizations (USVs) are classified into three main categories (simple, complex, and with frequency jumps). Simple USVs are identified as syllables with a unidirectional frequency with a duration that is shorter (short) or longer than 5 ms (flat, upward, downward). Complex syllables are characterized by frequency modulations in more than one direction (modulated) or with inclusion of one or more additional frequency components (complex). Frequency jumps are identified as syllables showing one (one jump) or several frequency jumps (frequency jump) with (mixed) or without sound during the transition.

Figure 2. During sexual interaction, pheromones emitted by the female are detected by the olfactory bulb, which then stimulates the neural circuitry underlying sexual behavior. Signals are transmitted to the medial amygdala (MeA), bed nucleus of stria terminalis (BNST), and medial preoptic area (MPOA), the key hypothalamic region involved in the motivation to vocalize. The production of ultrasonic vocalizations is controlled by motor circuitry, not illustrated in this figure, including the motor cortex and periaqueductal grey, as reported by Arriaga *et al.* (2012). Exposure to endocrine disrupting compounds (EDC) can interfere with the emission of courtship vocalizations through changes in sex steroid levels or their signaling pathways.

Figure 3. Effect of experience on ultrasonic vocalizations (USVs) in a mouse line lacking the neural androgen receptor. Behavioral analyses were performed on control and mutant littermates

that were castrated and supplemented with testosterone implants to normalize their hormonal levels, as previously described (Raskin et al., 2012). Males were tested when naïve and two weeks after mating with sexually receptive females. **A)** Percentage of naïve and sexually experienced males of both genotypes emitting vocalizations for 5 min in the presence of a sexually receptive female. **B-C)** Total number (**B**) and duration (**C**) of USVs emitted by control and mutant littermates that vocalized during the test. Data are expressed as the means \pm S.E.M. of 9 to 12 males per genotype. Two-way ANOVA shows an effect of genotype ($F_{(1,13)} = 8.62$, $p = 0.012$) and experience ($F_{(1,13)} = 13.75$, $p = 0.002$) on the number of USVs. There was also an effect of genotype ($F_{(1,13)} = 12.95$, $p = 0.003$) and experience ($F_{(1,13)} = 17.71$, $p = 0.001$) on the duration of USVs. *Post hoc* analyses showed differences between the two genotypes for both naïve and experienced animals; * $p < 0.05$, ** $p < 0.01$.

Table 1

Effects of exposure to endocrine disrupting compounds on ultrasonic vocalizations. AR: androgen receptor; DEHP: di-(2-ethylhexyl)phthalate; ED: embryonic day; ER: estrogen receptor; BNST: bed nucleus of stria terminalis; MeA: medial amygdala; MPOA: medial preoptic area; PCB: polychlorinated biphenyls; PND: postnatal day.

	Reference	Specie	Exposure period	Route	Doses	Age at analyses	Analyses of ultrasonic vocalizations (USVs)	Analyses of sexual behavior, hormone levels or hormone-sensitive tissues
Developmental	Pocock et al. 2002	Wistar rats	Gestation and lactation until PND21	Oral (diet)	Octylphenol: 100–250 mg/kg/day	PND7, PND12, and PND220 for males	Pups isolated from mothers: USVs emitted at PND7 were reduced in number and duration for both sexes. Adults in social interaction: no effects on USVs (data not shown).	No effect on testicular weight at PND19. Heavier testes at adulthood.
	Bell et al. 2016	Sprague–Dawley rats	Prenatal exposure at ED16, ED18, and ED20. Juvenile exposure at PND24, 26, and 28.	Injection (ip)	PCB (Arochlor1221) Prenatal exposure: 1 mg/kg Juvenile exposure: 1 mg/kg	PND30 to PND39, and adulthood	Adolescents in play interaction: prenatal exposure was associated with fewer USVs post-play, but only for females. Adults in the presence of receptive females: USVs production was not affected by 1 mg/kg prenatal exposure.	In preference tests, prenatal exposure to PCB reduce the amount of time spent near the no-hormone treat stimulus animal.
	Krishnan et al. 2018	Sprague–Dawley rats	ED8 to ED18	Injection (ip)	PCB (Arochlor1221): 1 mg/kg Vinclozolin: 1 mg/kg	Adult males of F2 generation	In the presence of receptive females: Vinclozolin: total calls were emitted at significantly lower rates by males of the paternal lineage. PCB: males of the paternal lineage had lower power/intensity than the vehicle group, narrower bandwidth than the vinclozolin group, and lower mean frequency than both the vehicle and vinclozolin groups.	Mating tests: higher frequency of intromission and decreased latency to ejaculation for males of both PC and vinclozolin groups. Lower estradiol levels in the vinclozolin-group. Lower circulating testosterone levels in the PCB-group.
Adult	Dombret et al. 2017	C57BL/6J mice	Adulthood	Oral (diet)	DEHP: 0.5, 5, 50 µg/kg/d	Adults: 4-6 weeks after exposure	Reduced total number and duration of USVs at 50 µg/kg/d The frequency of eight syllables of nine was reduced after chronic exposure to DEHP-50, whereas the number of short and flat USVs decreased in the DEHP-0.5 and DEHP-5 groups. The percentage of USVs per category remained unchanged in the DEHP-50 group, but was altered in the DEHP-0.5 and DEHP-5 groups relative to the vehicle group.	Increased latency to intromission and ejaculation. Reduced attraction of receptive females in three-chamber test (for 5 and 50 µg/kg/d groups). Unchanged levels of testosterone. Down-regulation of neural AR at 5 and 50 µg/kg/d and unaffected expression levels of ERα in the neuro circuitry underlying sexual behavior (MeA, BNST, MPOA).
	Capela et al. 2018	C57BL/6J mice	Adulthood	Oral (diet)	Nonylphenol: 0.5, 5, 50 µg/kg/d	Adults: 4-6 weeks after exposure	Increased total number and duration at 5 µg/kg/d, with increased syllable number for one jump and frequency jump syllables. Increased total duration was also observed for flat, upward, modulated, one jump, and frequency jump syllables.	Increased duration of mating, number of mounts, intromissions and thrusts. Unchanged levels of testosterone. Modifications in the number of AR- and ERα-immunoreactive cells in the MeA, BNST and MPO.

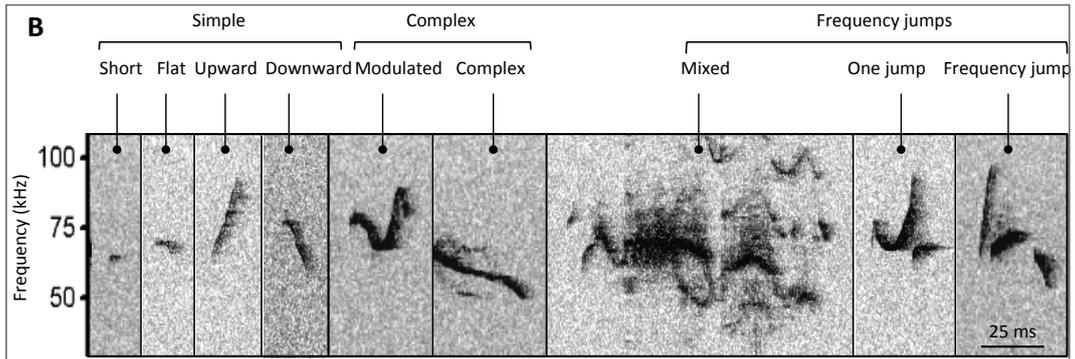


Figure 1

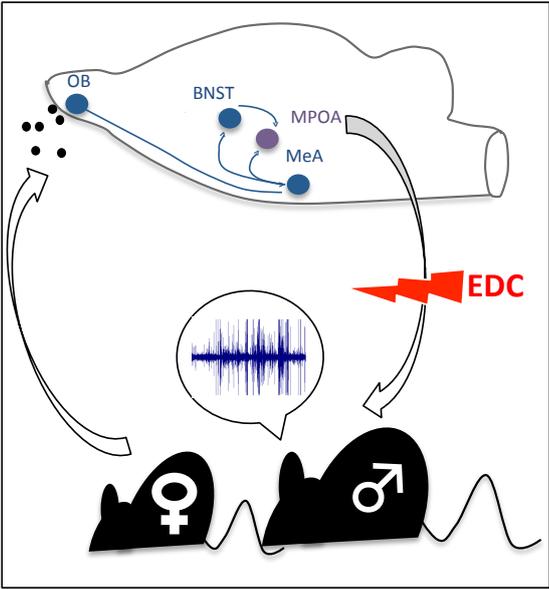


Figure 2

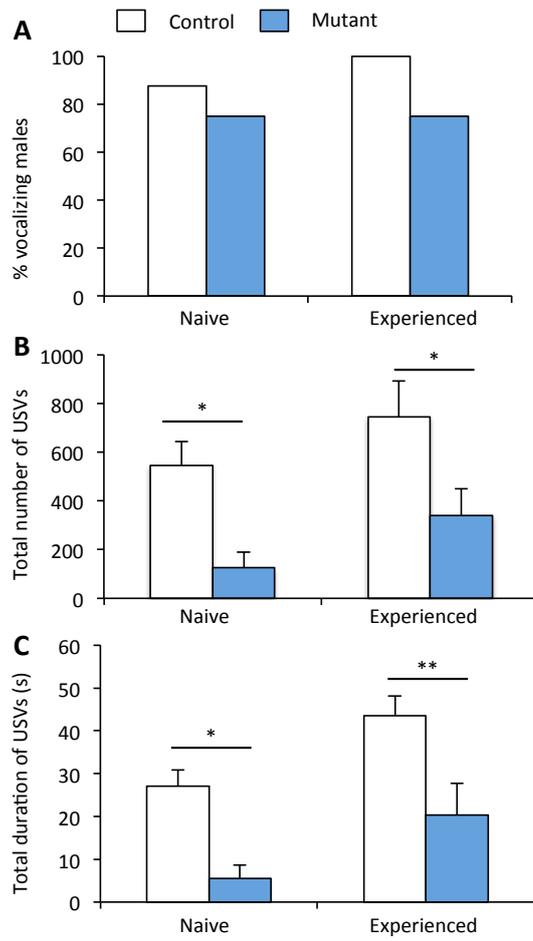


Figure 3