

Received:
19 April 2017
Revised:
29 September 2017
Accepted:
24 October 2017

Yawning: a cue and a signal

Cite as: A. Moyaho,
A. Flores Urbina,
E. Monjaraz Guzmán,
O. Walusinski. Yawning: a cue
and a signal.
Heliyon 3 (2017) e00437.
doi: [10.1016/j.heliyon.2017.e00437](https://doi.org/10.1016/j.heliyon.2017.e00437)

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Abstract

Yawning behaviour has been associated with a variety of physiological and social events and a number of corresponding functions have been attributed to it. Non-directed (self-directed behaviour) and directed yawning (display behaviour) might nonetheless encompass all expressions of yawning, although it is difficult to differentiate one type from the other in a social context. Here we analysed more fully the data from a study in which four combinations of sensory cues were presented to pairs of either cage mate or stranger rats. The aim of the re-analysis was to demonstrate that non-directed and directed yawning might be identified by their distinctive functions. All pairs of rats used olfactory cues to recognise each other as stranger or cage mate companions, but only stranger rats used auditory cues to detect and respond to each other's yawning. Increasing defecation rates (i.e. an index of emotional reactivity) inhibited yawning in cage mate rats such that yawning frequency reflected each rat's physiological state. These results suggest that non-directed yawning functions as a cue in cage mate rats and directed yawning as a signal in stranger rats. We hypothesize that cue yawning might be a regulatory act that animals perform to adjust muscle tone for a coordinated change of state. Signal yawning might indicate the physiological capacity of rats in male-male conflicts.

Keywords: Neuroscience, Zoology

1. Introduction

Yawning—a deep inspiration with mouth opening and slow expiration—is a widespread behaviour amongst all vertebrates. The behavioural repertoire of organisms distantly related to each other such as fish, birds and mammals includes yawning (Walusinski and Deputte, 2004), although most of our understanding about the prominent features of yawning (e.g. contagion) came from studies in humans (for an extensive review, see Provine, 2005). In spite of this ubiquity and the relative ease with which yawning can be identified and yawns counted, the biological significance of yawning is unknown. The growing number of functions linked to yawning reflects the diversity of phenomena to which it has been associated. All of these functions, however, may be grouped as physiological (true or rest yawn) or communicative (e.g. emotion or tension yawn).

Most of the physiological hypotheses of yawning's significance are based on a restorative function (i.e. homeostatic mechanism), including the opening of Eustachian tubes (Laskiewicz, 1953), equilibrium of CO₂ and/or O₂ levels in the blood (Sauer and Sauer, 1967; Provine et al., 1987b), prevention of atelectasis (Cahill, 1978), correction of imbalance in cerebral oxidative metabolism (Lehmann, 1979), proper articulation of the temporomandibular joint (de Vries et al., 1982), evacuation of potentially infectious substances from the tonsils (McKenzie, 1994), brain thermoregulation (Gallup, 2007), stimulation of the carotid body by compression (Matikainen and Elo, 2008), auto-regulation of the locomotor system (Bertolucci, 2011), and more recently, a process of switching the default mode network to the attentional system through the capacity of yawning to increase circulation of cerebrospinal fluid (Walusinski, 2014). However, none of the physiological hypotheses have received sufficient empirical support.

Yawning behaviour has also been associated with the presence of physiological disorders. For example, yawning may indicate encephalitis (Wilson, 1940), haemorrhage (Nash, 1942), motion sickness (Graybiel and Knepton, 1976; Matsangas and McCauley, 2014), the beginning of hypoglycaemia, which is a prodromal sign of vaso-vagal reaction (Cronin, 1988), and stress (Kubota et al., 2014). It is still unknown whether there is a cause-effect relationship between yawning and these physiological disorders, or if they are simply coincidental manifestations of a regulatory function.

Yawning frequency has also been associated with increased cholinergic and peptidergic activity (Dourish and Cooper, 1990), and decreased dopaminergic activity (Dourish and Cooper, 1990). These associations may contribute to the understanding of the immediate mechanisms involved in yawning behaviour, but they hardly say anything about the biological meaning of yawning.

Most communicative hypotheses about yawning come from observational studies performed in non-human primates in whom teeth-bearing during yawning has led to the suggestion that this behaviour reinforces dominant identity rather than signalling a threat (Deputte, 1994). Accordingly, yawning has been compared with intimidating displays that dominant males usually show to subordinate males (Troisi et al., 1990; Walusinski and Deputte, 2004; Zucker et al., 1998). In contrast, Sauer and Sauer (1967) proposed that yawning might induce relaxation of social tension. Yawning has also been described as a displacement activity—an unexpected display by an animal that appears to be engaged in other activity (Delius, 1967; Troisi, 2002)—and as an indication of changes in behavioural state (Provine et al., 1987a; Greco et al., 1993). There is also little experimental evidence to support these hypotheses.

What makes the identification of yawning with a function even more complicated is the fact that the physiological correlates of yawning, which might be used to support a given hypothesis, may produce contrasting effects. Thus, stimulation or inhibition of yawning has frequently been associated with changes in stress/anxiety, from the mild stress produced by exploration (e.g. a rat placed in a new environment) or vigilance, to the strong stress produced by fear (e.g. response-dependent punishment). For example, a new environment inhibits a rat's yawning, as compared with the increase observed following repetitive exposure to the same observation setup (Moyaho and Valencia, 2010). Low levels of vigilance have been linked to yawning occurrence in young human adults (Provine and Hamernik, 1986). Moreover, a mild shock to the feet in rats failed to suppress the yawning which preceded it (Moyaho and Valencia, 2010). Although these results clearly show a relationship between yawning and stress/anxiety, they do not indicate a consistent pattern; this may be partly due to variables which cannot be quantitatively controlled, making it difficult to determine precise cause-effect relationships. The use of quantitative variables such as defecation rate—an indication of emotional reaction to external stimuli which reflects the physiological state of the body—may clarify the relation of yawning with factors that cause stress to an animal, and thus help to recognise yawning functions.

Equating physiological and communicative functions of yawning with non-social and social contexts respectively is inaccurate since both forms may occur in a social context. A more precise identification would be with non-directed yawning (i.e. performed without the intention of eliciting a response from another individual) and directed yawning (i.e. performed with the intention of eliciting a response from another individual) respectively (Hall and Devore, 1965; Anderson, 2010). Nonetheless, it is not yet clear how to distinguish them in a social context. If a given animal yawns in front of conspecifics, this might be considered as directed yawning (i.e. display behaviour). Conversely, if an animal yawns without being watched by conspecifics, this could be taken as an instance of non-directed

yawning (i.e. self-directed behaviour). Nevertheless, it would be unjustified to assume that other sensory modalities do not also participate in the detection of yawning; therefore, apparently non-directed yawning could indeed be directed. Recently, [Moyaho et al. \(2015\)](#) scored yawning rates in cage mate and stranger rats that were exposed in pairs either to auditory, olfactory or visual cues. The authors found that only stranger rats showed auditory contagious yawning, although cage mate rats showed correlated defecation rates, a possible indication of emotional empathy. These differences between cage mate and stranger rats confirm the participation of sensory cues other than visual cues in yawning, and suggest that the differential effect of the treatments applied to the two groups of rats might help in recognising yawning functions.

This study tested the hypothesis that the differences which cage mate and stranger rats showed in terms of yawning reflect two different functions of yawning. For this purpose, the study re-analysed the data obtained by [Moyaho et al. \(2015\)](#) using probabilistic and statistical models.

2. Material and methods

[Moyaho et al. \(2015\)](#) previously described the material and methods they used for the experiments. Briefly, they used 188 adult male rats which were selected for high-yawning (HY) in Sprague-Dawley rats ([Urbá-Holmgren et al., 1990](#)). The experiments involved HY rats living either in the same cage (i.e. cage mate rats) or in different cages (i.e. stranger rats), and that were exposed to the presence or absence of olfactory and visual cues to determine their effect on yawning behaviour. [Moyaho et al. \(2015\)](#) placed each pair of rats in adjacent glass cages (19 cm², 10 cm height, 3 mm thickness) with each cage having a sliding lid and 24 holes (0.5 cm diameter) in its single acrylic side (3 mm thickness). The opposite side was 0.7 cm shorter than the other sides to allow each rat to breathe. The authors also used four acrylic sheets (19 cm width, 30 cm height, 3 mm thickness) as dividers between adjacent cages; two of the sheets were clear to provide visual contact and two were opaque to prevent it. One clear divider and one opaque divider each had 24 holes, identical to those in the glass cages. Thus, four testing scenarios were created: (1) visual and olfactory communication (VOC, perforated clear sheets), (2) visual communication (VC, non-perforated clear sheets), (3) olfactory communication (OC, perforated opaque sheets), and (4) neither visual nor olfactory communication (NVOC, non-perforated opaque sheets). Two of the 8 test situations (i.e. four cage mate pairs and four stranger pairs) were run each day. [Moyaho et al. \(2015\)](#) found that the acrylic sheets did not prevent the rats from hearing each other, and so they assessed the role of auditory cues in contagious yawning using trios of HY male rats. Two of the three rats, which were stranger rats, were placed in adjacent observation cages with olfactory communication; one of them was injected with kanamycin (K) to make it deaf, while the other was

injected with saline solution (S). The third rat was a sibling (SS) of the S rat and was placed in an observation cage next to an empty observation cage; the two cages had olfactory communication.

Because defecation is a validated measurement of emotional reactivity (Boissy, 1995; Ramos and Mormede, 1998), Moyaho et al. (2015) measured the rats' defecation rate before (pre-test emotional reactivity) and after (post-test emotional reactivity) the recording of yawning to assess the physiological response of the rats to the test situations. The difference between pre-test and post-test defecation rates—weighted by the rat's body mass—served as a comparative measure of emotional reactivity to the test situations. Observers recorded yawning behaviour for an hour. Yawning was defined as a single deep inspiration with mouth opening and slow expiration (Walusinski and Deputte, 2004).

2.1. Statistical analysis

Generalized linear mixed models (GLMMs) and model simplification were used to analyse the data. Model simplification was used to obtain minimal adequate models, which are preferred over full models according to the principle of parsimony (Crawley, 2007). Likelihood ratio tests were used for model selection, and either Wald t or Wald z for making specific comparisons after a minimal adequate model was obtained.

A joint distribution analysis was carried out to determine whether the paired rats simultaneously took particular sets of yawning values (e.g. one and one yawns, one and two yawns, one and three yawns, and so on). For this purpose, the yawning values per minute of the rats of each pair were separated and then combined into two groups. The allocation to each group did not vary from pair to pair because it was determined relative to a fixed point in the observation setting. Moreover, because the rats of each pair were randomly allocated to each observation cage, the separation of yawning values into two groups gave rise to two random sets of data per test situation. The frequencies of the combinations of corresponding yawning values were then listed in a two-way table. Then a bootstrap sampling procedure—10,000 samples using MATLAB (R2009b)—was run using a handle function which computed a statistic. The statistic calculated observed and expected averages from the combinations assuming independence between the two sets of yawning values. The magnitude of the difference between the observed and expected averages determined the degree of departure from independence between the two sets of yawning values; the larger the departure from independence the greater the frequency of occurrence of a given combination of yawning values between the two rats. Finally, the set of differences was ranked in ascending order and then the lower and upper quantiles were obtained (i.e. 95% confidence interval (CI)).

A statistical analysis was applied to determine whether one rat responded (i.e. yawned) to the other rat's yawning. For this purpose a geometric distribution model was used (Baclawski, 2008) to estimate how many times one rat had to yawn before obtaining a yawning response from the other rat. The null model assumed that responding or not responding to one rat's yawning was equally likely. This analysis was applied to the pairs and trios of rats. It was decided to include the trios' yawning in the analysis because one rat of each trio, a sibling of one of the other two rats, was alone next to an empty cage and therefore its frequency of yawning provided a control against which to compare the other rats' yawning.

The rats in each pair were separated into rats with more yawns and rats with fewer yawns, assuming that these differences were correlated with a superior-inferior relation in terms of yawning capacity.

To investigate whether the yawning of one rat reflected the rat's own physiological state or the other rat's behaviour, the relationship between yawning rates and defecation rates was analyzed using three types of statistical analysis. 1. Ordinary least-squared regression models were used to investigate the relation between average yawning rates and average defecation rates. 2. Logistic regression was used to assess the relationship between the probability of yawning and defecation rates. For this purpose, the rats from each pair were divided into rats with more and fewer yawns, and defecation rates were transformed to $2 \times \arcsin(\sqrt{\cdot})$. Then GLMMs were applied in which yawning (one for more yawns and zero for fewer yawns) was the response variable. A binomial distribution of errors was used and the data were analysed separately for cage mate and stranger rats. Block—a factor in the experiment—was a random-effects variable, and test situations, pre-test and post-test defecation rates were the explanatory variables. 3. A Pearson's product-moment correlation followed by principal axis regression was applied to investigate whether one rat's yawning rates correlated with the rat's own defecation rates or with the other rat's defecation rates. Accordingly, the difference in yawning rates between the rats was regressed on their corresponding difference in defecation rates, which were transformed to $2 \times \arcsin(\sqrt{\cdot})$.

All statistical analyses were carried out using R software (R Development Core Team, 2012), except where noted otherwise, and p values < 0.05 were considered statistically significant.

3. Results

Fig. 1 shows that the degree of dependency between the yawning values of each pair of rats varied with the sensory cue to which they were exposed. The average dependency of yawning values was greater in the paired rats exposed to olfactory communication (OC) than in the pairs exposed to the other test situations, as the corresponding CI (16.5, 14.81–19.12; mean with 95% CI) does not overlap the

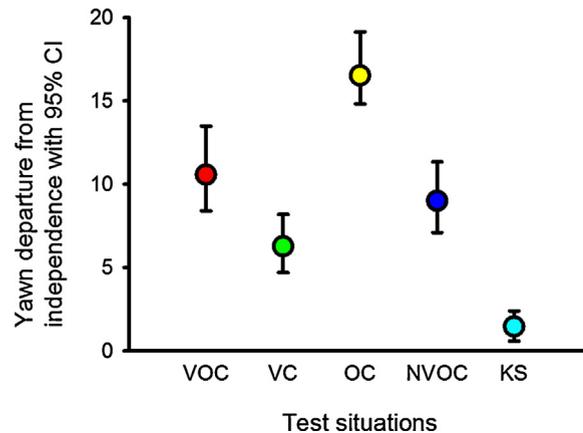
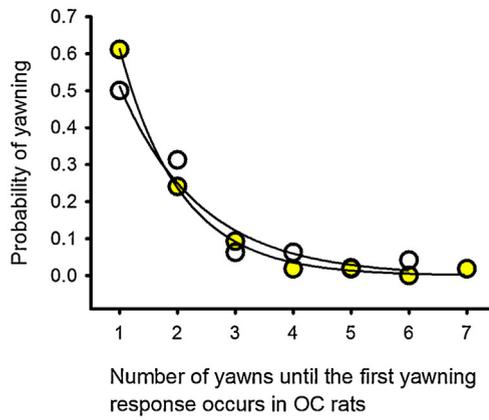


Fig. 1. Olfactory cues facilitate conjoint yawning in pairs of rats. Each circle represents the mean difference—with 95% CI—between the observed and expected combination of yawning values of each pair of rats. The data were collected from 15 to 18 pairs of male rats exposed to visual and olfactory communication (VOC), visual communication (VC), olfactory communication (OC), neither visual nor olfactory communication (NVOC), and olfactory communication between kanamycin- and saline-treated rats (KS).

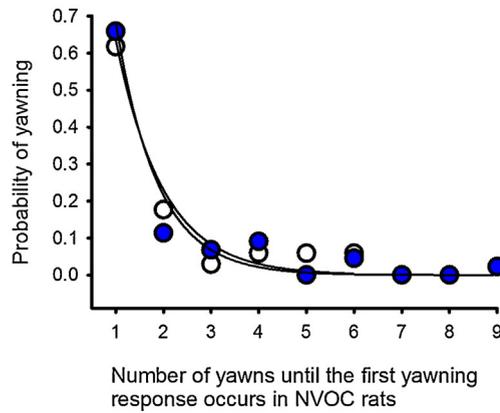
neither CI, that of rats exposed to visual and olfactory communication (VOC). For such dependency of yawning values to occur, both rats likely perceived auditory cues, as demonstrated by the low departure from independence shown by KS pairs (i.e. pairs of kanamycin- and saline-treated rats).

Fig. 2 shows that half the time or more, one rat responded to a single yawn by the other rat, although this response rapidly declined. In addition to this general trend, significant differences occurred between cage mate and stranger rats (Table 1). Fig. 2A shows that cage mate OC rats with more yawns responded (i.e. yawned) to the rats with fewer yawns, at a rate (0.51 ± 0.03 , estimate \pm s. e.) which did not differ from what was expected to occur for an unbiased response (0.5; t -test, $t_5 = 0.31$, $p = 0.77$, $n = 6$). In contrast, as Fig. 2A shows, stranger OC rats with more yawns responded to the rats with fewer yawns, at a rate (0.61 ± 0.01) which was greater than expected by chance ($t_6 = 12.21$, $p = 0.001$, $n = 7$). Interestingly, Fig. 2B shows that both stranger and cage mate rats with more yawns and neither visual nor olfactory communication (NVOC) responded to their companion rats with fewer yawns, at rates (0.68 ± 0.04 , and 0.64 ± 0.04 , respectively) greater than expected by chance alone ($t_8 = 4.35$, $p = 0.001$, $n = 9$ and $t_5 = 3.06$, $p = 0.014$, $n = 6$). Thus auditory cues are not sufficient for cage mate NVOC rats to distinguish between familiar and unfamiliar conspecifics because they responded as if the next cage rat was a stranger rat. Fig. 2C shows that the rate at which saline-treated rats (S) responded (0.67 ± 0.01) to the yawning of kanamycin-treated rats (K) was greater than 0.5 ($t_6 = 8.94$, $p = 0.001$, $n = 7$). In contrast their siblings (SS)—located on the opposite side of the observation set up and separated by a wooden divider—responded to the yawning of the same K rats at a rate (0.52 ± 0.05) that

A



B



C

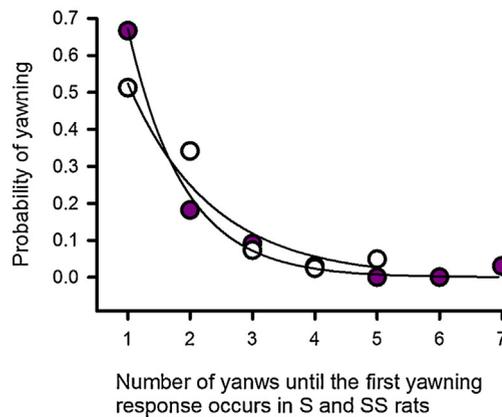


Fig. 2. Yawning communication between stranger rats exposed to auditory and olfactory cues. (A) Full and empty circles represent respectively the probability of yawning in stranger and cage mate OC rats

did not differ from 0.5 ($t_4 = 0.47$, $p = 0.659$, $n = 5$). K rats hardly responded to either S or SS rats' yawning (Table 1). Neither OC nor NVOC rats with fewer yawns significantly responded to the rats with more yawns (Table 1). This lack of response could not be explained by a small number of yawns alone because neither OC nor NVOC stranger rats with fewer yawns differed in yawning frequency from SS rats ($F_{1,25} = 0.14$, $p = 0.87$, $n = 9-10$), which nonetheless yawned at a rate not different from 0.5 (i.e. an unbiased response; Fig. 2C).

Fig. 3A shows that a comparatively low rate of yawning produced by visual cues is associated with a comparatively high level of emotional reactivity in cage mate rats, as average yawning rate per test situation significantly decreased with average defecation rate ($r^2 = 0.94$, $F_{1,2} = 32.64$, $p = 0.029$; $n = 4$). Moreover, the probability of yawning decreased with pre-test defecation rate in the same cage mate rats ($z = -2.19$, $p = 0.028$; $n = 68$; Table 2 and Table 3). Therefore, while visual cues might produce stress in pairs of cage mate rats and reduce their yawning, a slight pre-test reaction to a novel environment might predict that a rat will yawn more frequently than its companion. Stranger rats did not show any significant relationship between average yawning per test situation and average defecation rate ($r^2 = 0.02$, $F_{1,2} = 0.05$, $p = 0.844$; $n = 4$). Nor did they show any significant association between the probability of yawning and either pre-test or post-test defecation rate (Table 4). Fig. 3B shows a significant negative association between the difference in yawning rate and the corresponding difference in defecation rate in OC rats ($r = -0.91$, $t_7 = 6.09$, $p = 0.000$, $n = 9$). This association indicates that if, for example, the rats of each pair were to choose between yawning in response to their own physiological state—reflected through their defecation rate—or the other rat's physiological state, they would prefer the former, even though they probably perceive each other's physiological state since their defecation rates are correlated (Moyaho et al., 2015). A similar association was also shown by cage mate VOC rats ($t_7 = 2.98$, $p = 0.02$, $n = 9$; Fig. 4), but not by the rats in the other test situations, indicating that olfactory cues are necessary for this association to occur in cage mate rats.

Average yawning rate per pair increased, albeit moderately, with defecation rate in stranger OC rats ($r^2 = 0.63$, $F_{1,6} = 10.4$, $p = 0.018$, $n = 8$). However, as Fig. 5A illustrates, this increase did not reflect the rats' own defecation rates because the yawning rate of stranger OC rats with fewer yawns positively correlated with the

with more yawns in response to the yawning of their companions with fewer yawns (see Fig. 1 for abbreviations). (B) As in A, except that the circles represent the probability of yawning in NVOC rats. (C) Full and empty circles represent respectively the probability of yawning in stranger saline-treated rats (S) and S' sibling rats (SS) in response to the yawning of kanamycin-treated rats. The lines in A-C are the probability density functions which best fit the data according to a geometric distribution. The probability of yawning is statistically different between the function fitted in A and also C (t-test, $t_{11} = 2.6$, $P = 0.024$ and $t_{10} = 2.72$, $P = 0.021$, respectively).

Table 1. Summary of the fit of geometric distributions to the time steps a rat would wait for the other rat to yawn, in different test situations.

	Test situation	Probability of yawning	Mean \pm SDM	n
Cage mate rats	OC +*	0.51 \pm 0.04	1.95 \pm 0.56**	6
	OC –	0.24 \pm 0.02	4.17 \pm 0.71	26
	NVOC +	0.64 \pm 0.04	1.57 \pm 0.39	6
	NVOC –	0.20 \pm 0.02	5.03 \pm 1.16	15
Stranger rats	OC +	0.61 \pm 0.01	1.63 \pm 0.38	7
	OC –	0.28 \pm 0.03	3.52 \pm 0.90	11
	NVOC +	0.68 \pm 0.04	1.46 \pm 0.27	9
	NVOC –	0.26 \pm 0.02	3.85 \pm 0.80	17
	SK	0.67 \pm 0.02	1.49 \pm 0.32	7
	KS	0.30 \pm 0.02	3.36 \pm 0.89	10
	SSK	0.52 \pm 0.05	1.91 \pm 0.59	5
	KSS	0.32 \pm 0.03	3.08 \pm 0.73	12

*Plus or minus signs refer to test situations with rats from a pair with more and fewer yawns, respectively; test situations without a sign refer to trios of rats. The pairs of rats were exposed to the following sensory cues: OC, olfactory communication; NVOC, neither visual nor olfactory communication. The trios consisted of two rats (saline(S)- and kanamycin(K)-treated rats) with olfactory communication, and a saline treated rat sibling of the S rat (SS) which was placed next to an empty cage with olfactory communication. Thus, the abbreviations for the trios refer to: SK, yawning of an S rat in response to a K rat; KS, yawning of a K rat in response to an S rat; SSK, yawning of an SS rat in response to a K rat; KSS, yawning of a K rat in response to an SS rat. The probability of yawning is expressed as the mean \pm standard deviation of the mean (SDM). **Average time steps (min) one rat would wait for the other rat to respond. n refers to the number of trials.

defecation rate of the rats with more yawns ($r = 0.81$, $t_6 = 3.39$, $p = 0.015$, $n = 8$). The converse was not true; the yawning rates of stranger OC rats with more yawns did not correlate with the defecation rate of the rats with fewer yawns ($r = -0.21$, $t_6 = -0.54$, $p = 0.61$, $n = 8$).

This effect between stranger rats depended on olfactory cues as illustrated in Fig. 5B which shows a significant positive association between yawning rate difference and defecation rate difference in K and S rats ($r = 0.81$, $t_8 = -3.98$, $p = 0.004$, $n = 10$). This association means that if K and S rats were given a choice between yawning in response to their own physiological state and the other rat's physiological state, they would prefer the latter. A significant association like this one was not found between S and SS rats ($r = -0.16$, $t_8 = -0.47$, $p = 0.645$, $n = 10$).

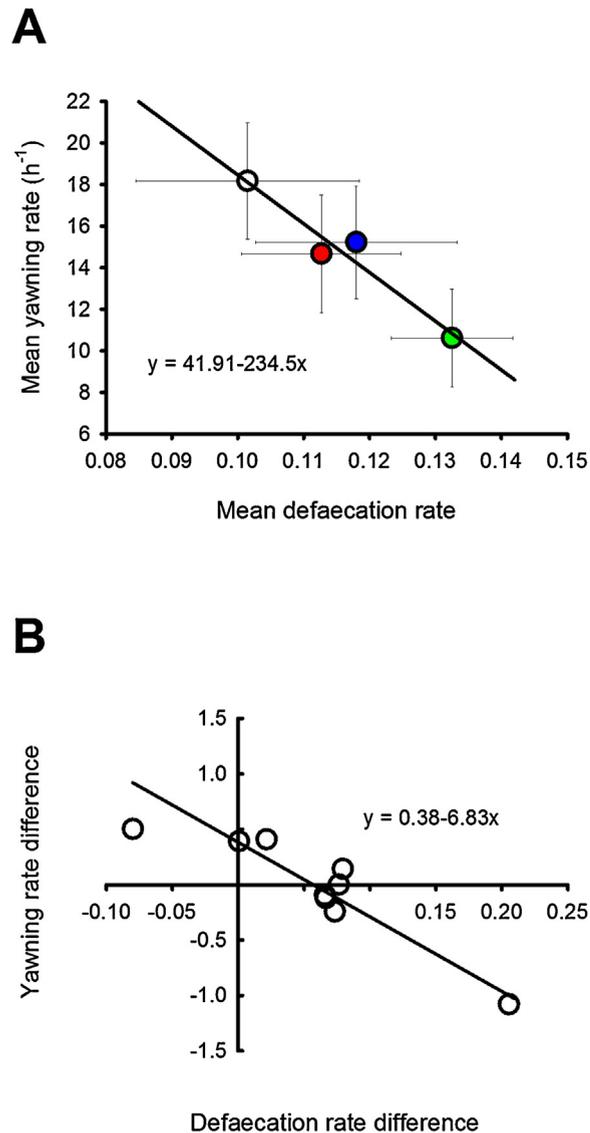


Fig. 3. Yawning reflects the physiological state of cage mate rats. **(A)** Statistically significant decrease—the line and equation represent the best fit using regression analysis ($P = 0.029$)—in the mean rate of yawning \pm SEM (vertical lines) in 9 pairs of cage mate male rats as a function of their mean defaecation rate ($2 \times \arcsine(\sqrt{\quad})$ transformed data) \pm SEM (horizontal lines). Circles from left to right correspond to OC, VOC, NVOC, and VC rats (see Fig. 1 for abbreviations). **(B)** Statistically significant association ($P < 0.001$) between yawning rate difference and defaecation rate difference in 9 dyads of cage mate OC male rats. Yawning rate difference was obtained by subtracting the yawning rate of one rat from the yawning rate of the other rat. Defaecation rate difference was obtained in a similar way, except that the subtraction was reversed so that while one rat had positive yawning rates and negative defaecation rates, the other rat had the converse.

Table 2. Model simplification of the fit of GLMMs to the data on the relationship between the probability of yawning and defecation rate in pairs of cage mate rats.

Step	Simplification	d.f.	logLik	Deviance change (χ^2)	P
Maximal model	_____	17	-36.53		
1	trt ¹ + dr1 + dr2+ dr1:dr2 + dr1: trt + dr2:trt	14	-40.39	7.73	0.051
2	trt + dr1 + dr2	7	-43.70	6.60	0.471
3	dr1 + dr2	4	-44.08	0.75	0.859
4	dr1	3	-44.10	0.04	0.833
5	intercept	2	-47.13	6.06	0.014

¹trt, treatment (pairs of rats exposed to 1. Visual and olfactory communication (VOC), 2. Visual communication (VC), 3. Olfactory communication (OC), 4. Neither visual nor olfactory communication (NVOC)); dr1, pre-test defecation rate; dr2, post-test defecation rate. The plus and colon symbols denote, respectively, inclusion of an explanatory variable and interaction between two or more explanatory variables in the model. P values refer to the change in statistical significance when the relevant model terms are removed.

4. Discussion

The purpose of this study was to re-analyse a database from a previous study, which suggested that the differential response of cage mate and stranger rats to a combination of sensory cues could be used to identify the function of yawning. With the re-analysis, directed and non-directed yawning were respectively identified with the response of stranger and cage mate rats to the combination of olfactory and auditory cues. The rats used the olfactory cues to discriminate between cage mate and stranger rats, and the auditory cues to detect and respond to each other's yawning.

Table 3. Summary of the minimal adequate model as obtained in Table 2.

Random effects	Variance			
Block	7.65e-12			
Residual	2.76e-6			
Fixed effects	Value	SE ¹	Wald Z	P
Intercept	0.49	0.32	1.53	0.125
Pre-test defecation rate	-18.43	8.40	-2.19	0.028

¹Standard error of the estimated value of the relevant parameter.

Table 4. Model simplification of the fit of GLMMs to the data on the relationship between the probability of yawning and defecation rate in pairs of stranger rats.

Step	Simplification	d.f.	logLik	Deviance change (χ^2)	P
Maximal model	_____	17	-36.45		
1	trt ¹ + dr1 + dr2+ dr1:dr2 + dr1: trt + dr2:trt	14	-42.97	13.04	0.004

¹trt, treatment (see Table 2 for abbreviations). The minimal adequate model was the maximal model, although none of the explanatory variables significantly affected the probability of yawning ($P > 0.05$).

Yawning frequency could be a reliable indication of a rat's physiological state, as yawning rate and defecation rate (an index of emotional reactivity) showed a consistent negative association in cage mate rats, so that frequent yawning was a genuine indication (i.e. a cue) of a low-arousal state of calm. This interpretation agrees with a steady increase in yawning frequency recorded over several days in HY male rats placed daily in the same observation cages. This increase presumably happened because the rats became acquainted with the test condition (Moyaho and Valencia, 2010), which might have progressively caused less stress. While average yawning rate per test situation decreased with average defecation rate in cage mate rats—so that the greater the uncertainty about the next cage rat's identity the greater the defecation rate (i.e., NVOC rats)—the rat of each pair that surpassed the other in yawning frequency was the one that tended to show the lower pre-test

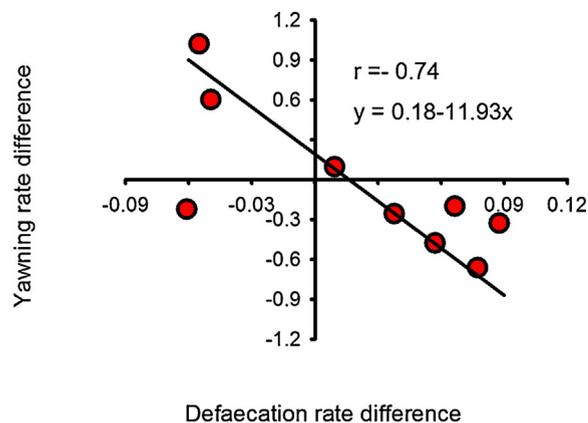


Fig. 4. The relationship between yawning rate difference and defecation rate difference in 9 pairs of cage mate rats exposed to visual and olfactory communication (VOC). Each circle represents the yawning rate difference for a given defecation rate difference. Yawning rate (log transformed data) difference was obtained subtracting the yawning rate of one rat from the other rat. Defecation rate ($2 \times \arcsin \sqrt{\quad}$) difference was obtained in a similar way, except that the subtraction was reversed so that whereas one rat had positive yawning rates and negative defecation rates, the other rat had the converse. The line and equation represent the best fit according to principal axis regression.

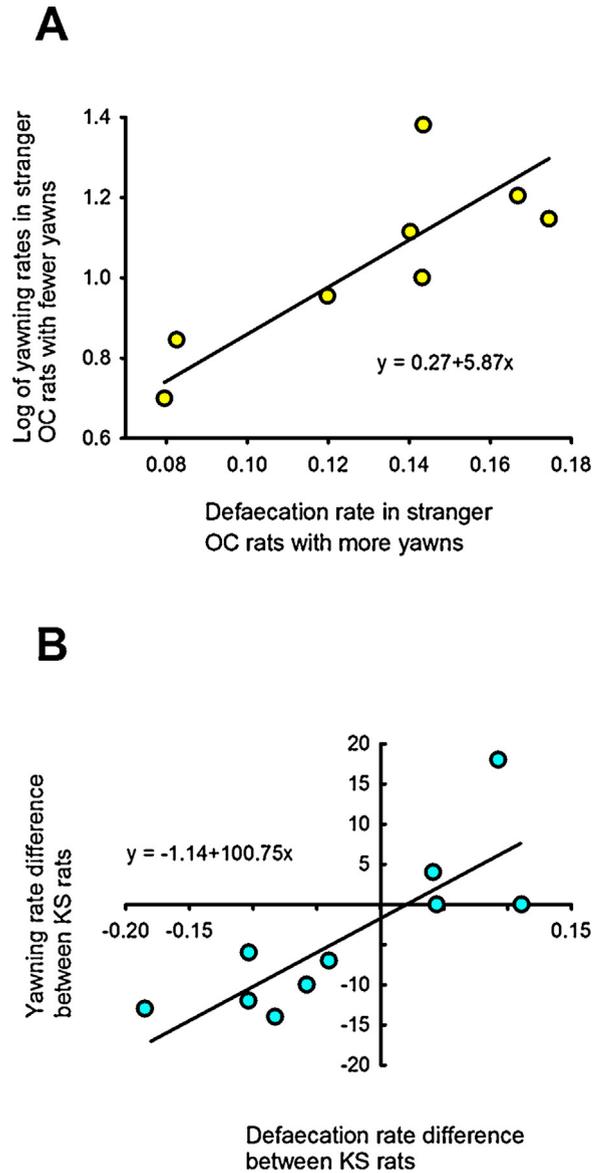


Fig. 5. The relationship between yawning and defaecation rates in stranger rats exposed to olfactory communication. **(A)** Statistically significant relation ($P = 0.015$) between yawning rates in stranger OC rats with fewer yawns and defaecation rates in the rats with more yawns. Here one pair was removed from the analysis because the rats showed equal yawning rates. **(B)** Statistically significant association ($P = 0.004$) between yawning rate difference and defaecation rate difference in 10 pairs of kanamycin- and saline-treated rats (KS). The lines and equations in **A** and **B** represent the best fit according to a principal axis regression analysis.

defaecation rate. Thus, somehow the relative yawning frequency of each pair of cage mate rats reflected a difference acquired before the test situation to which they were exposed.

The existence of a negative association between yawning and defecation rate in cage mate rats contrasts with the lack of such an association in stranger rats. Nonetheless, the pairs of stranger rats with olfactory communication yawned more frequently with higher defecation rates, although the relation was moderate. A thorough analysis of this relation revealed the existence of a communicatory effect within each pair of rats, since the yawning of the rats with fewer yawns positively correlated with the defecation rate of the rats with more yawns. Similarly, the yawning of saline-treated rats showed a positive association with the defecation rate of the kanamycin-treated rat. Therefore, stranger rats affected one another's behaviour most likely through yawning.

The ways in which cage mate and stranger rats used yawning behaviour in response to olfactory and auditory cues are aligned with the distinction between a cue and a signal (Maynard Smith and Harper, 2003). A cue is a feature of the world, animate or inanimate, that an animal can use as a guide to future action (Hasson, 1994), and a signal is an act or structure that an animal uses to change the behaviour of another animal. The effect that the act produces, fosters its evolution, and the evolution of the receiver's response promotes its effectiveness (Maynard Smith and Harper, 2003). In the case of cage mate rats with olfactory communication, there must have been quick individual recognition based on familiarity and previously established dominance hierarchies, which has been suggested to occur in many animals (Bradbury and Vehrencamp, 1998). Because there was no physical contact between the rats, volatile chemicals likely played a role in mutual recognition, and auditory signals likely played a role in coordinating responses. It is unlikely, however, that the yawning observed in the rats exposed to olfactory communication was a direct response to volatile chemicals, although this could be the case in male bats that yawned during social interactions (Gebhard, 1997; as cited by Voigt-Heucke et al., 2010). In any case, cage mate rats probably recognized each other readily and adjusted their behaviour accordingly; in this context of familiarity, no conflict regarding dominant-subordinate roles should exist between the rats because a dominance hierarchy has already been established. If so, cage mate rats would mostly be irresponsive to each other's behaviour, as evidenced by the finding that they would choose to yawn in association with their own defecation rate. Therefore, this type of yawning can be referred to as a cue which may have evolved into a regulatory act associated with a rat's physiological state.

We specifically propose that cue yawning is a motor act used to diminish variation in muscle tone. This function is reasonable as a hypothesis because of the type and large number of muscles involved in yawning (approximately 54 in humans; Walusinski, 2004). These muscles participate in proprioception and interoception by conveying information used to indicate how an individual feels (Craig, 2003; Walusinski, 2006). Moreover, it is known that the main function of proprioceptive

reflexes is to adjust the motor output according to the biomechanical state of the body and limbs; thereby a compensating mechanism for the intrinsic variability of such output is achieved (Pearson and Gordon, 2000). Thus if yawning is an involuntary motor act, it would be part of the proprioceptive reflexes involved in decreasing the intrinsic variability of muscle tone—something like tuning up a musical instrument—so as to ensure operation efficiency.

The hypothesis proposed by Bertolucci (2011)—that yawning (he did not distinguish between cue yawning and signal yawning) increases the level of tone necessary for activity—explains the frequency of yawning observed, for example, following awakening, but not the frequency preceding sleep onset, when it is known that most mammals also yawn. The hypothesis that cue yawning reduces the variation in muscle tone accounts for the increase of yawning observed before a change of state, either from resting to activity or from activity to resting. Moreover, if the hypothesis is correct, intra- and inter-individual variation in yawning rates would reflect the magnitude of muscle tone variation in each individual, and thus the number of yawns required to decrease it. Thus, the subjective ratings of feeling associated with yawning can vary from unpleasant to pleasant according to the reduction of muscle tone variation achieved by an individual. As a consequence of a pleasant state following yawning, an individual may show unconcern or indifference (Baenninger and Greco, 1991), and thus the possibility of agonistic behaviour (e.g. dispute, conflict, etc.) decreases.

In contrast to cage mate rats, an encounter between stranger rats most likely initiates competitive scent marking so that each rat can determine the other's individual features as well as its ability to defend territory and resources (Hurst and Beynon, 2004). Scent marking and counter-marking are advertising strategies commonly used by rodents to establish control over resources (Hurst and Beynon, 2004); the chemicals released function as a reliable mechanism by which rats can provide information about their strain, sex, individual identity (Brennan and Kendrick, 2006), and current reproductive and health status (Hurst and Beynon, 2004). This creates a context of conflict in which most individuals would attempt to settle any dispute involving territory and resource defence without a fight by using signals to persuade each other to flee (Bradbury and Vehrencamp, 2011).

The findings of the present study agree with the existence of conflict—and concomitant attempts at resolution—between stranger rats, because those with more yawns and under olfactory (or auditory) communication yawned in response to the yawning of those rats with fewer yawns; the rats with more yawns probably played a dominant role and were more motivated. In fact, previous studies have revealed that a context of frequent male-male encounters might promote yawning (Moyaho et al., 2009). Moreover, the rats with more yawns might also have high levels of steroid hormones. There is a significant positive association between

yawning and penile erections (Holmgren et al., 1985; Moyaho et al., 2015) that depends on the action of steroid hormones (Melis et al., 1994; Phoenix and Chambers, 1982), which also facilitate aggressive behaviour. Therefore, the rat with more yawns of a pair might also have more testosterone, and hence better odds of winning an eventual fight. Thus, yawning frequency might be an honest signal of physiological capacity in stranger rats (Moyaho et al., 2015). Nevertheless, for yawning to be an honest signal, it should entail a cost. Although no studies have assessed the costs of yawning, its motor complexity suggests that it could be physiologically exhausting—a large number of muscles are recruited—and also a risky act, because whenever an individual yawns it becomes vulnerable to predation, as it exaggeratedly opens its mouth and closes its eyes. In addition to these potential costs, there is evidence that males have low survival rates caused by testosterone-dependent behavioural traits which are necessary to achieve a dominant status (Sinervo et al., 2000).

Yawning behaviour could have evolved through the ritualization of a cue resulting from changes in physiological state (Maynard Smith and Harper, 2003). Such a cue might be linked to vomeronasal olfaction, which is involved in intra-specific chemical communication (Bradbury and Vehrencamp, 1998) in many mammals and reptiles. For example, when mammals contact urine or secretions, many of them (e.g. antelopes, felines) raise their heads and retract the upper lip to facilitate perception of odorants. This is a behavioural pattern called flehmen (Bradbury and Vehrencamp, 1998). Indeed, there is a type of yawning in chimpanzees in which the lips are funnelled outwards that resembles flehmen (Vick and Paukner, 2009). Similarly, a pumping mechanism in hamsters facilitates perception of volatile chemicals from the vomeronasal organ (Meredith et al., 1980). Also, the occurrence of mouth gaping in rattlesnakes, which is an analogue behaviour to flehmen or yawning in mammals, increases when snakes are exposed to conspecific skin chemicals (Graves and Duvall, 1983), once again apparently to facilitate vomeronasal olfaction.

This type of chemical communication is frequently associated with reproduction in both mammals and reptiles (Meredith and Fernandez-Fewell, 1994). In fact, the vomeronasal organ is larger in males than in females (Halpern, 1987), a size difference that parallels the sexual dimorphism in yawning behaviour, which in several species is more frequent in males than females (Bertrand, 1969; Deputte, 1994; Goy and Resko, 1972; Hadidian, 1980; Hall and Devore, 1965; Redican, 1975). It is unlikely, however, that yawning behaviour is currently used to stimulate vomeronasal olfaction, given that some species which yawn have lost the vomeronasal organ (e.g. fish and birds; Bertmar, 1981), and given the exaggeration of mouth opening. Nonetheless, the ancestral origin of yawning and its broad presence in animals are aligned with the evolution of the vomeronasal organ, including the lachrymal ducts (Bertmar, 1981), the content of which is frequently

released with yawning. This coincidence, as well as the association with spontaneous penile erections strongly suggests that yawning behaviour arose from the ritualization of pre-existing cues involved in perceiving stimulating chemosignals in a mating context.

5. Conclusion

To date, there is no convincing explanation for the biological significance of yawning, which remains an elusive issue. The variety of contexts associated with yawning does not necessarily mean yawning has several functions. Instead, current literature suggests that mammals, at least, tend to yawn in two major situations: when an individual most likely directs its yawning to a conspecific, and when an individual does not direct its yawning to any conspecific, because it is either alone or not in the line of sight of the conspecific. In effect, as the analysis of data presented here shows, there seem to be two types of yawning; the one shown by cage mate rats is an act reflecting the physiological state of the body, and therefore might be considered as a cue. We propose that an individual yawns because it needs to adjust muscle tone (i.e. decrease the variation), so that the muscles can appropriately work together when a change of state is needed. The other type of yawning, which stranger rats showed, involves the interaction of at least two individuals in which the yawning of one of them affects or is affected by the other individual's behaviour. Further studies are necessary to clarify the precise direction of the effect; nonetheless, we propose that this type of yawning might function as a signal in male-male conflicts and probably involves the physiological capacity of the contestants. The consistent association of yawning with penile erections and testosterone seems to support this hypothesis.

In summary, the findings of this study provide a framework in which earlier categorizations for the function of yawning converge into two expressions: cue yawning, which may function as a regulatory act of the level of muscle tone variation; and signal yawning, which may function as a physiological capacity signal.

Declarations

Author contribution statement

Alejandro Moyaho: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Amira Flores Urbina, Eduardo Monjaraz Guzmán: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Olivier Walusinski: Conceived and designed the experiments; Wrote the paper.

Funding statement

A. Moyaho, A. Flores Urbina, and E. Monjaraz Guzmán were supported by Vicerrectoría de Investigación y Estudios de Posgrado, Benemérita Universidad Autónoma de Puebla.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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