Auditory disturbances promote temporal clustering of yawning and stretching in small groups of budgerigars (*Melopsittacus undulatus*)

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Abstract

Yawning may serve both social and non-social functions. When budgerigars (Melopsittacus undulatus) are briefly held, simulating capture by a predator, the temporal pattern of yawning changes. When this species is observed in a naturalistic setting (undisturbed flock), yawning and also stretching, a related behavior, are mildly contagious. Based on these findings, we hypothesized that a stressful event would be followed by the clustering of these behaviors in a group of birds, which may be facilitated both by a standard pattern of responding to a startling stressor and also contagion. In this study, we measured yawning and stretching in four-bird groups following a non-specific stressor (loud white noise) for a period of one hour, determining whether auditory disturbances alter the timing and frequency of these behaviors. Our results show that stretching, and to a lesser degree yawning, were non-randomly clumped in time following the auditory disturbances, indicating that the temporal clustering is sensitive to, and enhanced by, environmental stressors while in small groups. No decrease in yawning such as found after handling stress was observed immediately after the loud noise, but a similar increase in yawning 20 minutes after was observed. Future research is required to tease apart the roles of behavioral contagion and a time-setting effect following a startle in this species. This research is of interest because of the potential role that temporal clumping of yawning and stretching could play in the collective detection of, and/or response to, local disturbances or predation threats.

Keywords: arousal; contagious behavior; collective behavior; vigilance

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Introduction

Yawning is a ubiquitous vertebrate behavior that has been hard to characterize functionally, primarily because there are numerous eliciting stimuli, including general stress. The relationship between social stress and yawning is documented in humans and non-human primates (Greco, Baenninger, & Govern, 1993; Troisi, Aurelli, Schino, Rinaldi, & De Angelis, 1990), and may occur in other mammals. The effect of physiological stress on yawning in rodents was also studied, indicating that when exposed to foot shock, yawning is initially low but then gradually increases (Moyaho & Valencia, 2002). Related to hypotheses generated by these studies, much comparative research supports the view that yawning is involved in the maintenance of arousal (for review, see Baenninger, 1997), and is also related to changes in state or activity (Provine, 1996; 2005). Although studies have failed to identify yawn-associated increases in cortical arousal as measured by EEG (reviewed by Guggisberg, Mathis, Schnider, & Hess, 2010), recent research shows that distinct brain temperature reductions occur following yawning in rats (Shoup-Knox, Gallup, Gallup, & McNay, 2010), and this cooling effect has been hypothesized to promote arousal. In addition, research on humans, chimpanzees and rats shows that yawning is associated with behavioral arousal, as measured by modified activity or increased locomotion (Baenninger, Binkley, & Baenninger, 1996; Gallup, Miller, & Clark, 2011; Giganti, Hayes, Akilesh, & Salzarulo, 2002; Vick & Paukner, 2009). Therefore, it seems likely that the association between stress and yawning is connected to a general state of arousal and activity.

To date, only in budgerigars (*Melopsittacus undulatus*) is there experimental evidence that yawning is related to stress in a non-mammalian vertebrate (Miller, Gallup, Vogel, & Clark, 2010). In that study, handling of a bird simulated capture by a predator, and, after release, behavioral responses were measured over three 20-minute time blocks (Miller, Gallup, Vogel, & Clark, 2010). In comparison to control periods, yawning was delayed and infrequent in the first 20 minutes following release, but then significantly increased in frequency during the next 20 minutes — a temporal pattern similar to that described in rodents (see Moyaho & Valencia, 2002). A follow-up experiment showed that a bird's under-wing temperature after handling was negatively related to its latency to yawn (accounting for over 38% of variance in this response). These results suggest that stress-induced yawning may be associated with hyperthermia. At the same time, the delay in yawning may be an adaptive suppression when a predator is still near. Thus observed patterns of yawning may represent compromises between conflicting adaptive responses, namely a need to thermoregulate versus a need to freeze in face of a potential threat.

Recent observational research provides evidence that both yawning and stretching are socially contagious in these birds (M. L. Miller, A. C. Gallup, A. R. Vogel, S. M. Vicario, & A. B. Clark, in review), showing tight temporal clustering and even matching of these behaviors in unmanipulated flocks (~20 individuals). It is hypothesized that contagion may function to coordinate arousal and collective movement within groups, and therefore we expect it to be enhanced by the presence of an environmental threat or disturbance.

Methods

The goal of this study was to determine the effect of an auditory stressor on the temporal

pattern of yawning and stretching in budgerigars. To accomplish this, each of four groups of budgerigars (four birds per group, n = 16 birds) was independently exposed to both a loud white noise sound (~60 dB) and a control condition (no sound), each condition presented on separate days at 1300h. White noise was selected as the stressor because this sudden, novel noise clearly startled the birds (personal observation: M. L. Miller), but was not related to any threat they had experienced. Animals were selected from a flock of birds kept in a large aviary $(1.8 \times 1.8 \times 1.8 \text{ m})$ and were placed in groups of four, in a wire-mesh testing cage $(0.4 \times 0.3 \times 0.3 \times 1.8 \times 1.8$ m), 24 hours prior to the first trial (Binghamton University IACUC protocol #629-08). Over the next two days, each group was exposed to both the white noise² (30 seconds) and the control conditions, one trial-condition per day in randomized order (two groups experienced the white noise condition first). The wire cage was moved into a separate room one hour before the trial started. The camera was started when the animals were moved, but the first hour of the video file was ignored (acclimation). The sound was triggered remotely from outside the room, so no other disturbance occurred at the start of the trial. Birds were videotaped for 60 minutes following each trial-condition. A team of two reviewers that were blind to the condition recorded the time of all yawns and stretches by each bird on all tapes. From this, we determined, for each bird, the latencies to perform each behavior and the latency to move (either laterally while perched or by initiating flight), following both conditions. As in the study of handling stress (Miller et al. 2010) we also broke the hour into three 20-minute time blocks to test for a change in the temporal pattern of these behaviors following the stimulus. For statistical tests, ANOVAs were used to compare latencies or frequencies of a given behavior between conditions. These statistical models treated group as a between-subjects factor, and condition and time interval (where appropriate) as within-subjects factors.

The temporal clustering of each behavior following either an auditory disturbance or control period was evaluated using the runs tests (see Sokal & Rohlf, 1995, pp. 797 - 799). Using the same methodology from our previous study (Miller et al., in review), patterns of yawning and stretching were analyzed in the following way (described for yawns). Each one-hour taping session was broken into 180 twenty-second bins, and the number of observed yawns in each bin was calculated. A run was composed of consecutive bins identified as either having at least one yawn (1), or having no yawns (0). For instance, '111000' contains two runs of three 20second periods. During the first run, there was at least one yawn in each interval (111). Thus, there was no distinction between bins with one versus multiple yawns. The second run represents three intervals totaling one minute with no yawns (000). For each group, a separate runs test was performed for each behavior (yawns and stretches) during each of the two conditions. The runs test quantifies clustering by comparing the observed number of runs to the expected number of runs, standardized to a normal distribution. For this reason, consecutive behaviors performed by the same bird within a twenty-second interval were excluded, so that clumping would not be attributed to a single individual. The output from this test is a Z score, and negative scores indicate greater clumping rather than dispersion. Combined probability tests were then used to determine the probability of non-random clumping across the four trials of a given condition (each behavior separately) (see Sokal and Rohlf, 1995, p. 795). The combined probability test is used to evaluate a common null hypothesis that was independently tested multiple times.

² Downloaded from: http://www.simplynoise.com/

Results

A total of 49 yawns were observed in the hour following the white noise stimulus (3.1 \pm 0.81 yawns/bird), which was not significantly different from the 33 observed during the control condition (2.1 \pm 1.08 yawns/bird) ($F_{1,12} = 3.728$, p = 0.077) (Fig 1A). There was a nearly significant time interval \times condition interaction ($F_{2,24} = 3.149$, p = 0.061) that became significant when group was removed from the model ($F_{2,30} = 3.41$, p = 0.046). Post hoc tests reincorporating group show that yawning frequency did not differ initially after exposure to the loud noise, as compared with the control condition ($F_{1,12} = 0.128$, p = 0.727), but yawning was more frequent during the second 20 minutes following noise compared with the control ($F_{1,12} = 10.787$, p = 0.007) (Fig 1A). Although there was no main effect for group ($F_{3,12} = 1.216$, p = 0.346), this factor significantly interacted with time ($F_{6,24} = 3.663$, p = 0.01). Lastly, there was no difference between conditions in the latency to first yawn ($F_{1,12} = 2.568$, p = 0.134, Fig 1A), and no effects of group on this measurement (p values > 0.05).

In one of the four 4-bird groups, yawns were significantly clustered during the full hour following the experimental stimulus (Group 2: Z = -2.977, p < 0.003; see Fig 2A). The three other groups had similarly negative Z scores following the auditory disturbance, but no group showed significant clustering of yawns during the control condition (average Z score \pm 95% CI = 0.57 \pm 0.22). Overall, there was significant clustering across the four noise trials (the four independent groups) (combined probability test: χ^2_8 = 17.98, p = 0.021), but not across the four independent control trials (χ^2_8 = 4.59, p = 0.8). When each 20-min interval was considered separately, significant clumping was observed for at least one group during only the second and third intervals, but not the first interval (Fig 2B). When tested with combined probability tests, however, no single interval, for both noise and control conditions, was significantly clumped (p values > 0.05).

Seventy-eight stretches were observed after the noise stimulus $(4.9 \pm 1.58 \text{ stretches/bird})$, which was not significantly different from the 60 observed after the control condition $(3.8 \pm 1.62 \text{ stretches/bird})$, $F_{1,12} = 1.533$, p = 0.239) (Fig 1B). Unlike yawning, the white noise did not affect the overall distribution of this behavior across the three 20-minute intervals (Fig 1B). Similar to yawning, however, group significantly interacted with time $(F_{6,24} = 7.535, p < 0.001)$ and condition $(F_{3,12} = 3.817, p = 0.039)$, although there was no main effect of group $(F_{3,12} = 1.297, p = 0.32)$. The latency to first stretch did not differ between the two conditions $(F_{1,12} = 0.207, p = 0.657)$, and group did not affect this measurement (p values > 0.05) (Fig 1B).

Stretching was significantly clumped in three of the four groups following the white noise condition (average Z score \pm 95% CI = -2.84 \pm 1.32, three p values < 0.05), but not during any of the control trials (average Z score \pm 95% CI = -0.76 \pm 0.99, all p values > 0.05) (Fig 2C). Based on the combined probability test, stretches were significantly clumped during the noise trials but not the control trials (noise: χ^2_8 = 48.09, p < 0.001; control: χ^2_8 = 10.07, p = 0.260). When trials were broken into 20-minute intervals, the most apparent clustering was observed during the first 20-minutes, immediately after the white noise, with gradual decreases observed in the subsequent intervals (Fig 2D). Based on the combined probability tests across noise trials, the first two intervals were significant for clumping (36.65 < χ^2_8 < 42.68, p values < 0.005), while there was a trend for increased clumping in the third interval (χ^2_8 = 13.88, p = 0.085). None of the intervals from the control conditions were significantly clumped (χ^2_8 < 7.79, p values > 0.05).

Discussion

Our findings show that stretches, and yawns to a lesser degree, were non-randomly clumped in time following the auditory disturbances, indicating that the degree of temporal clustering is sensitive to environmental stimuli. Two potential explanations emerge from the increased temporal clustering following a loud noise. First, the behaviors themselves may have become more contagious among flock-mates following the auditory stressor. Alternatively, clumping may have been a result of individuals having a similar behavioral response to this stimulus, thus promoting synchronization that was not socially influenced. Given the interaction between time and group, the latter explanation is unlikely, since if non-social synchronization was responsible for all results, one would expect the time courses of all groups to be similar. Furthermore the lack of any difference in latency measures between conditions and the extension of temporal clustering into the later two time blocks provides support that this pattern resulted largely from behavioral contagion, and not result of an initial, non-social coordination.

We did not find any temporal clumping under control conditions like that documented in our observational study of larger flocks (~20 individuals) (Miller et al., in review), but this could be due to a number of factors including differences in group size and separation, disruptions in rest-activity cycle associated with experimental testing, and the limited time frame in which recordings occurred. While observational studies of contagious yawning have also been presented in other species, such as gelada baboons (*Theropithecus gelada*: Palagi, Leone, Mancini & Ferrari, 2009), unequivocal experimental evidence of this effect is limited to humans (Provine, 1989; Platek, Critton, Myers & Gallup, 2003) and chimpanzees (*Pan troglodytes*: Anderson, Myowa-Yamakoshi & Matsuzawa 2004; Campbell, Carter, Proctor, Eisenberg & de Waal, 2009). Further research is needed to determine whether yawning and stretching can be contagiously triggered among budgerigars in a controlled experiment, as well as identify the various factors associated with the temporal clustering of these behaviors in this and other species.

The proximate function of stretching is not well understood, but, like yawning, is probably a homeostatic behavior, possibly involved in behavioral arousal. In fact, when mildly disturbed (e.g., person entering a room without close approach) or motivated by external events including food, budgerigars commonly stretch and then initiate flight (personal observation: A. B. Clark, A. C. Gallup, M. L. Miller & A. R. Vogel), suggesting that stretching is involved in preparation for movement. This makes it a possible signal of activity, and similar to yawning, the temporal clustering of stretching could coordinate changes in a group's activity-level or state.

When comparing the results from this study to those of Miller et al. (2010), one obvious difference is that the latency to yawn in the current study did not differ between white noise and control conditions. This suggests that, unlike handling restraint, the auditory disturbance did not suppress yawning. Because handling stress simulates an encounter with a predator, release is essentially "escape" and the subsequent inhibition in yawning may be an adaptive response to decrease detection by remaining very still. The white noise disturbance was presumably a non-specific stressor that was less threatening, producing mild vigilance without prolonged stillness. In fact, there was no difference in the latency to move after the white noise

compared to the control trials (noise: 185 ± 89 seconds, control: 106 ± 86 seconds, $F_{1,12} = 3.653$, p = 0.080). In contrast, we did observe an increase in yawning frequency 20 minutes following the stressor, which is comparable to that shown with handling (Miller et al., 2010).

Taken together with the previous report on handling stress (Miller et al., 2010), these findings suggest that yawning is adaptively suppressed or elicited, both socially or non-socially, depending on factors such as the immediacy of the threat and the degree of physiological stress experienced, although more precise measures of stress response are needed for confirmation. Furthermore, the tight social clumping of stretching after an auditory disturbance may be a collective response preparing the group for flight in the event that a more salient threat was presented. Since flight synchronization is believed to function in antipredator avoidance (Monas & Barta, 2011), we propose that future studies of group vigilance should consider yawning and stretching as potential variables of interest. Consistent with the view that contagious yawning evolved to coordinate arousal, which in turn may improve vigilance within the group (Gallup and Gallup, 2007), the temporal clumping of yawning and stretching witnessed after the auditory disturbance may serve to enhance collective detection of, and/or response to, local disturbances or predation threats.

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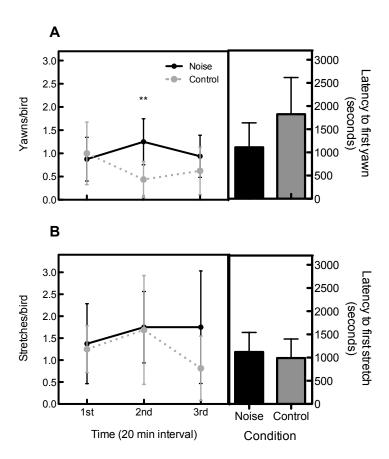
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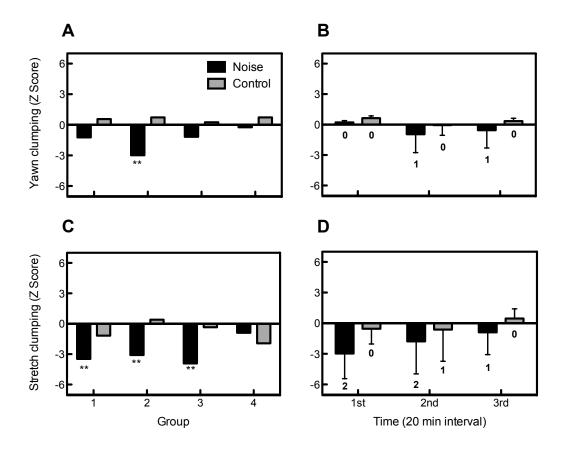
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Figure and figure caption



<u>Figure 1.</u> Loud white noise, an unnatural auditory disturbance, increased yawning 20-minutes following the stimulus. (A, B) Shown in the left panel are the number of yawns (A) and stretches (B) observed per bird during the three time blocks of the 1-hour session after each trial-condition (Mean \pm 95% CI) (**p <0.01) (left panel). The average latency before the first behavior of each bird is shown in the right panel.



<u>Figure 2.</u> Stretches, and to a lesser extent yawns, were more clumped in time after a white noise exposure. (**A**, **C**) Shown here are Z scores for yawns (**A**) and stretches (**C**) of each group during the noise and control trials (*p < 0.05 and **p < 0.01). (**B**, **D**) The average Z score (± 95% CI) during the first, second and third 20-minute intervals are shown for yawns (**B**) and stretches (**D**). Negative scores indicate greater clumping rather than dispersion. The numbers below each bar represent the number of trials that exhibited statistically significant clumping for those intervals (max = 4).