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# Phasic activity of the locus-coeruleus is not a mediator of the relationship between fitness and inhibition in college-aged adults

Madison C. Chandler [a,*], Amanda L. McGowan [a], Jan W. Brascamp [b], Matthew B. Pontifex [a]

[a] *Department of Kinesiology, Michigan State University, United States of America*
[b] *Department of Psychology, Michigan State University, United States of America*

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## ABSTRACT

Aerobic fitness is consistently and robustly associated with superior performance on assessments of cognitive control. One potential mechanism underlying this phenomenon is activation of the locus-coeruleus. Specifically, individuals with greater aerobic fitness may be better able to sustain engagement in a cognitively demanding task via a superior ability to meet the metabolic demands of this neural system. Accordingly, the present investigation examined 1) the relationship between aerobic fitness and phasic activation of the locus-coeruleus (indexed using pupillometry) and 2) the potential mediating influence of locus-coeruleus activity on the relationship between aerobic fitness and cognitive task performance. Participants performed an inhibition task while their pupillary responses were measured using an infrared eye tracker. A $VO_{2max}$ test was then performed to determine individuals' aerobic fitness levels. Consistent with previous research, higher levels of aerobic fitness were related to shorter reaction time. However, phasic activity of the locus-coeruleus did not mediate this relationship – nor did it relate to aerobic fitness level. These results suggest that aerobic fitness does not relate to differences in locus-coeruleus activity in the context of cognitive control in college-aged adults.

The health-related attribute of aerobic fitness – the body's ability to sustain aerobic physical activity (American College of Sports Medicine, 2018) – is related not only to optimal functioning of the body but also to optimal functioning of the brain. Across the lifespan, individuals who are more-aerobically-fit evidence superior cognitive processing in a variety of domains, including: academic achievement (see Donnelly et al., 2016 for a review), memory (Bunce and Murden, 2006; Pontifex et al., 2014b; Raine et al., 2013), and attention (Luque-Casado et al., 2016; Pontifex et al., 2009, 2012). However, this relationship presents perhaps most consistently and robustly in the area of cognitive control (Brassell et al., 2015; Buck et al., 2008; Hillman et al., 2009; Pontifex et al., 2011; Voss et al., 2011; Westfall et al., 2017, 2018), which refers to a class of operations that facilitate goal-oriented actions via planning, problem-solving, exerting control, and/or resisting impulses (Meyer and Kieras, 1997; Norman and Shallice, 1986). Meta-analysis of aerobic fitness training studies has suggested a general yet selective relationship such that the largest effects are observed for tasks requiring aspects of cognitive control (Colcombe and Kramer, 2003). Accordingly, the extant literature examining cross-sectional differences between higher- and lower-aerobically-fit individuals has primarily focused on this domain of cognition, and in particular, inhibitory aspects of cognitive control. Using inhibitory control tasks such as the Eriksen flanker task or Stroop tasks, investigations in this area have observed that higher-fit individuals exhibit superior performance (as indexed by greater response accuracy or faster reaction time) than their lower-fit counterparts (Alderman and Olson, 2014; Buck et al., 2008; Hillman et al., 2009; Huang et al., 2015; Mora-Gonzalez et al., 2020; Pontifex et al., 2011; Pontifex et al., 2014a; Westfall et al., 2018). Task-related parameters (stimulus duration speed, intertrial interval, etc.) have been speculated to contribute to whether the association manifests for response accuracy or reaction time. Although a more general relationship between fitness and task performance has been observed across trial types of these inhibitory control tasks, individuals with greater aerobic fitness appear better able to flexibly modulate cognitive control in response to more demanding situations and task instructions (Pontifex et al., 2011; Themanson et al., 2008; Westfall et al., 2017) and exhibit greater efficiency of neural networks associated with cognitive control (Voss et al., 2011).

As these high-level cognitive operations associated with cognitive control require substantial neural resources to maintain engagement, one potential mechanism for this superior level of performance is that individuals with greater levels of aerobic fitness may be better able to meet the metabolic demands imposed by sustained engagement.

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* Corresponding author at: Department of Kinesiology, 38 IM Sports Circle, Michigan State University, East Lansing, MI 48824-1049, United States of America.
*E-mail address:* chand138@msu.edu (M.C. Chandler).

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Accordingly, individuals with greater aerobic fitness may exhibit greater capacity to sustain activation of the locus-coeruleus—a collection of neurons in the brainstem involved with alertness and attention (Kinomura et al., 1996; Sara and Bouret, 2012). As the locus-coeruleus is involved in attentional filtering to facilitate task-relevant behaviors, this sustained activation may thus serve to optimize task performance. The purpose of the present investigation was therefore to examine the relationship between aerobic fitness and activation of the locus-coeruleus and to determine the mediating influence of locus-coeruleus activity.

The locus-coeruleus comprises a series of projections to the cerebral, cerebellar, and hippocampal cortices, providing the primary source of norepinephrine to these regions (Aston-Jones and Cohen, 2005). Research in non-human animal models has shown that the locus-coeruleus has direct inputs from the anterior cingulate cortex and the orbitofrontal cortex — both areas that are associated with cognitive control processes (Aston-Jones and Cohen, 2005). Interestingly, a robust body of literature suggests that there are aerobic-fitness-related differences in the activation of these cognitive control areas: specifically, higher-fit individuals exhibit increased activation of the anterior cingulate cortex and other neural areas associated with cognitive control relative to their lower-fit peers (Chaddock et al., 2012; Colcombe et al., 2004). Beyond these alterations in areas underlying cognitive control, non-human animal models demonstrate altered expression of a neuropeptide called galanin in the locus-coeruleus of exercising mice selectively bred to be high-capacity runners (Murray et al., 2010). Given the neuromodulatory role of galanin, the increased expression of this neuropeptide associated with elevated fitness may serve to alter the firing pattern of neurons in the locus-coeruleus and the associated release of norepinephrine (Wrenn and Crawley, 2001) – which in turn may enable superior performance on tasks requiring high-level cognitive operations. Because galanin expression in the locus-coeruleus appears to be age-dependent, with decreased galanin signaling as we age, the present study used college-aged adults to reduce potential confounds associated with age (de Bilbao et al., 1991).

During tasks requiring focused attention, neurons in the locus-coeruleus exhibit a characteristic pattern of phasic bursting that is coupled with the execution of a response to task-relevant stimuli (Aston-Jones and Cohen, 2005). In non-human animal models, direct recordings of cells in the locus-coeruleus have demonstrated that this phasic activity is positively associated with the latency of the behavioral response to target cues (Aston-Jones et al., 1994). Modern theoretical accounts of the locus-coeruleus suggest that the phasic activity of the locus-coeruleus and concurrent release of norepinephrine serves to facilitate the entrainment of other neural systems to limit responsiveness to irrelevant stimuli. This process thereby prevents spurious distractions and allows for facilitations in goal-directed behaviors and more rapid on-line adjustments in behavioral responses (Aston-Jones and Cohen, 2005; Bouret and Sara, 2005). Because neurons in the locus-coeruleus are involved not only in higher-order cognitive operations but also in vital neurophysiological functioning, the phasic activation of locus-coeruleus neurons has a high bioenergetic cost (Bekar et al., 2012; Mather and Harley, 2016). As such, sustained phasic activity of the locus-coeruleus is difficult to maintain. For example, sustained firing of neurons in the locus-coeruleus for a period of just 10 min results in decreased levels of norepinephrine in brain areas such as the prefrontal cortex – and it has been observed that locus-coeruleus neurons projecting to the prefrontal cortex are unable to maintain norepinephrine release over extended periods of time (Bellesi et al., 2016; Carter et al., 2010). Accordingly, individuals with greater levels of aerobic fitness may be better able to meet the metabolic demands imposed by such periods of high phasic activity of the locus-coeruleus, enabling superior behavioral performance.

Given the difficulty in directly assessing the activity of the locus-coeruleus, pupillometry has emerged as the predominant means of conducting noninvasive assessments (Gilzenrat et al., 2010; Murphy et al., 2014). Indeed, there appears to be a strong correlation between the firing rate of noradrenergic neurons in the locus-coeruleus and the moment-to-moment fluctuations in pupil size — with changes in neuronal activity reliably preceding changes in pupil diameter (Costa and Rudebeck, 2016; Joshi et al., 2016). Further, while changes in luminance provide a potent extrinsic driver of pupil size — such as in the case of the light-evoked pupillary reflex – when task-related stimuli are matched in novelty and luminance, task-evoked pupillary reactivity has been observed to modulate in response to the magnitude of mental load/conflict (Causse et al., 2016; Geva et al., 2013; Heitz et al., 2008; Scharinger et al., 2015). Within the context of an inhibitory control task such as the Stroop task, greater pupillary reactivity is observed in response to incongruent trials that require the suppression of the dominant response (Laeng et al., 2011; Scharinger et al., 2015). Conversely, mental fatigue – and corresponding cognitive task performance declines – coincides with lower levels of pupillary reactivity (Hopstaken et al., 2015).

Taken together, given the link between phasic activity of the locus-coeruleus and superior online attentional filtering leading to enhanced performance, the present investigation sought to determine the extent to which alterations in phasic activity of the locus-coeruleus might manifest across the continuum of aerobic fitness. With the well-established findings that higher-fit individuals evidence superior performance on cognitive control tasks and that non-human animal models demonstrate altered galanin expression in the locus-coeruleus (thereby influencing activation of the noradrenergic locus-coeruleus system), it was hypothesized that aerobic fitness would be related to greater phasic activity of the locus-coeruleus. Additionally, it was hypothesized that the phasic activity of the locus-coeruleus would mediate the relationship between aerobic fitness and behavioral performance on a test of cognitive control.

## 1. Method

### 1.1. Participants

The present investigation utilized a sample of 126 college-aged young adults (19.1 ± 1.1 years old) recruited from Michigan State University. All participants provided written informed consent in accordance with the Human Research Protection Program at Michigan State University and reported being free of any neurological disorder, psychological condition, previous history of head trauma, cardiovascular disease, physical disabilities, and indicated normal or corrected-to-normal vision. Demographic and fitness data for all participants are provided in Table 1.

### 1.2. Procedure

Using a cross-sectional design, participants visited the laboratory on a single day. Upon arrival, participants provided informed consent and completed both a health history and demographics questionnaire and the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas et al., 1992) to screen for any existing health issues that might be exacerbated by performing the aerobic fitness assessment. Participants were fitted

**Table 1**
Participant demographic and aerobic fitness characteristics (mean ± SD).

| Measure | All participants | [Range] |
|---|---|---|
| N | 126 (100 female) | |
| Age (years) | 19.1 ± 1.1 | [18–25] |
| Education (years) | 13.0 ± 1.2 | [12–17] |
| Nonwhite (%) | 30% | |
| Percent body fat (%) | 30.2 ± 7.9 | [13.1–54.3] |
| $VO_{2max}$ (ml/kg/min) | 42.3 ± 9.0 | [20.0–70.2] |
| $VO_{2max}$ percentile | 48.8 ± 33.5 | [3–97] |

*Note*: $VO_{2max}$ percentile based on normative values for $VO_{2max}$ from Shvartz and Reibold (1990).

with a wireless heart rate monitor and had their height, weight, and body composition measured using a stadiometer and an Omron HBF-510 digital scale, respectively. They were then seated in a chair equipped with a chin rest (Earthlite Avila II, Earthlite, LLC, Vista, CA) to keep their head in a fixed position and were provided an introduction to the inhibitory control task, with a goal of achieving at least 70% overall accuracy. If 70% overall accuracy was not achieved, the practice task was re-administered until this criterion was reached. Following the practice task, the EyeTribe infrared eye tracker (The Eye Tribe, Copenhagen, Denmark) was calibrated and participants were instructed to stare straight ahead at the screen, minimizing blinks, while black and white screens flashed in an alternating order to measure participants' light- and dark-evoked pupillary responses. One hundred sixty test trials of the task were then administered while participants' pupillary responses were measured using the eye tracker. Following completion of the cognitive task, participants' aerobic fitness was assessed using a maximal oxygen consumption ($VO_{2max}$) test.

### 1.2.1. Inhibitory control task

Inhibitory control was assessed using a letter version of the Eriksen flanker task (Eriksen & Eriksen and Eriksen, 1974; McGowan et al., 2019), which is classified as a behavioral response selection construct of the inhibition/suppression focus and the performance monitoring focus of cognitive control according to the NIMH Research Domain Criteria classification system. Participants were instructed to attend to an array of letters and to respond as accurately as possible to the centrally presented letter, ignoring the flanking stimuli. These arrays were either congruent (e.g., "M M M M M" or "N N N N N") or incongruent (e.g., "M M N M M" or "N N M N N"). Participants completed 40 practice trials followed by 160 test trials grouped into two blocks of 80 trials, each consisting of equiprobable congruency and directionality. For each block of trials, participants were presented with perceptually similar letter pairs (practice block: B – D, block 1: M – N, block 2: E – F) and were instructed to respond by pressing the button assigned to the centrally-presented target stimulus. To ensure a high degree of task difficulty, response compatibility was manipulated at the midpoint of each block by switching the stimulus-response mapping for each set of letters (e.g., left button press for "M" through the first 40 trials of block 1, then right button press for "M" through the last 40 trials of block 1). Flanking letters were presented 300 ms prior to target letter onset, and all five letters remained on the screen for a subsequent 100 ms (for a total stimulus duration of 400 ms) with a response window of 1000 ms and a variable inter-trial interval of 2300, 2400, 2500, 2600, or 2700 ms using PsychoPy, 1.85.2 (Peirce, 2009). Prior to completing the test block, participants completed a task introduction with a total of 40 practice trials in which the first 12 trials gradually decreased the target letter stimulus duration from 300 ms to 100 ms. All stimuli were 1.5 cm tall white block letters with a mean luminance of 129.1 $cd/m^2$. Stimuli were presented on a black background on an Asus 144 Hz LCD monitor (45.1 $cd/m^2$) at a distance of approximately 50 cm. Throughout the task, a central fixation dot was present on the screen and participants were instructed to minimize eye blinks.

### 1.2.2. Pupillometric measures

While participants performed the flanker task, pupillary activity was recorded using a table-mounted infrared eye tracker (The Eye Tribe, Copenhagen, Denmark) at a sampling rate of 60 Hz. Prior to beginning the cognitive task, the distance between the participant and the eye tracker was recorded and a 9-point calibration procedure was conducted to ensure quality and precision of the recorded signal. Pupil diameter was recorded in arbitrary units and then imported into EEGLAB (Delorme and Makeig, 2004), where it was then scaled to micrometers (McGowan et al., 2019). Dilation speed outliers and eyeblinks were removed including a 150 ms window prior to and following each artifact. After linear interpolation of discontinuities in the data, the pupillary response following the eyeblink and potential light reflexes were then corrected using Finite Impulse Response deconvolution and the continuous data was filtered using a 0.02 to 4 Hz bandpass Butterworth IIR filter (Knapen et al., 2016; McGowan et al., 2019). Phasic (task-evoked) pupillary reactivity was stimulus-locked using task-evoked epochs for correct trials from −1000 to 1800 ms around the stimulus and baseline corrected using the −200 to 0 ms pre-stimulus period (van Steenbergen and Band, 2013). To ensure the integrity of the signal, all epochs were visually inspected blind to fitness and congruency prior to computing mean waveforms across both left and right pupils (mean number of included trials: congruent = 105.2 ± 28.2 and incongruent = 102.6 ± 27.8). Phasic pupillary reactivity (as an index of phasic activity of the locus-coeruleus) was quantified as the peak pupil size within 0 to 1800 ms surrounding the stimulus (Murphy et al., 2011).

### 1.2.3. Aerobic fitness assessment

Following completion of the behavioral and pupillometric assessments, maximal oxygen consumption ($VO_{2max}$) was measured using a computerized indirect calorimetry system (ParvoMedics True Max 2400) to index aerobic fitness. Following a brief warm-up period, participants walked or ran on a motor-driven treadmill (Trackmaster TMX425C) at a constant speed with a 2.5% increase in grade every 2 min until maximal effort (i.e., the participant was no longer able to maintain the exercise intensity). A Polar heart rate monitor (Polar WearLink +31; Polar Electro, Finland) provided a continuous measure of heart rate throughout the test and ratings of perceived exertion values were assessed every 2 min with the OMNI scale (Pfeiffer et al., 2002). Relative peak oxygen consumption was expressed in milliliters per kilogram per minute (ml/kg/min) and was based on maximal effort as evidenced by attainment of at least two of four confidence criteria: 1) a plateau in oxygen consumption corresponding to an increase of less than 2 mL/kg/min despite an increase in workload, 2) a peak heart rate $\geq$ 190 bpm, 3) respiratory exchange ratio (RER) $\geq$ 1.1, and/or 4) ratings on the OMNI scale of perceived exertion >7 (McGowan et al., 2019; Pontifex et al., 2009). Aerobic fitness percentiles were extracted from normative data from Shvartz and Reibold (1990), accounting for both age and biological sex.

## 1.3. Statistical analysis

Hierarchical linear regression analyses were performed to determine the independent contribution of aerobic fitness (as assessed using $VO_{2max}$ percentile) for explaining variance in: mean reaction time, mean response accuracy, and phasic activity of the locus-coeruleus (as assessed using phasic pupillary reactivity) after accounting for statistically significant descriptive factors (i.e., Age, Biological Sex [0 = Female, 1 = Male], Race [0 = White, 1 = Nonwhite], and Percent Body Fat) and Congruency (0 = Congruent trials, 1 = Incongruent trials) (Pontifex et al., 2016; Pontifex et al., 2014b). For each analysis, a forward stepwise approach based upon Akaike Information Criterion (Akaike, 1974) was utilized to determine if including the interaction between aerobic fitness and task congruency improved the model fit.

All variables and analysis residuals were screened for normality and homoscedasticity using histograms, Q-Q plots, Shapiro-Wilk tests (Shapiro and Wilk, 1965), and Studentized Breusch-Pagan tests (Koenker, 1981). Although reaction time, response accuracy, and pupillary reactivity were not normally distributed, the analysis residuals were normally distributed and homoscedastic. Of note, all findings remained the same even when using logarithmic transformation. As such, the results presented below reflect use of the raw data. All data analyses were performed in R Version 4.0 (R Core Team, 2019) utilizing a familywise alpha level of $p = 0.05$. Given a sample size of 126 participants and a beta of 0.20 (i.e., 80% power), the present research design theoretically had sufficient sensitivity to detect the independent contribution of aerobic fitness if it exceeded an effect size of $f^2 = 0.06$ as computed using G*Power 3.1.2 selecting the "F tests" for "Linear multiple regression: Fixed model, $R^2$ increase" accounting for six total predictors (Faul et al., 2007).

## 2. Results

### 2.1. Initial relationships with aerobic fitness

#### 2.1.1. Reaction time

Hierarchical regression analysis indicated that individuals with greater aerobic fitness exhibited shorter reaction time (B = −0.35 [95% CI: −0.62 to −0.07], SE B = 0.14, Beta = −0.18) after accounting for the influence of demographic factors and task congruency, $R^2_{change} = 0.02$, $F_{change}(6, 243) = 6.1$, $p = 0.014$, $f^2 = 0.02$ [95% CI: 0.0 to 0.07] (see Fig. 1a). The inclusion of an interaction term between fitness and congruency did not improve model fit ($R^2_{change} < 0.01$, $p = 0.7$).

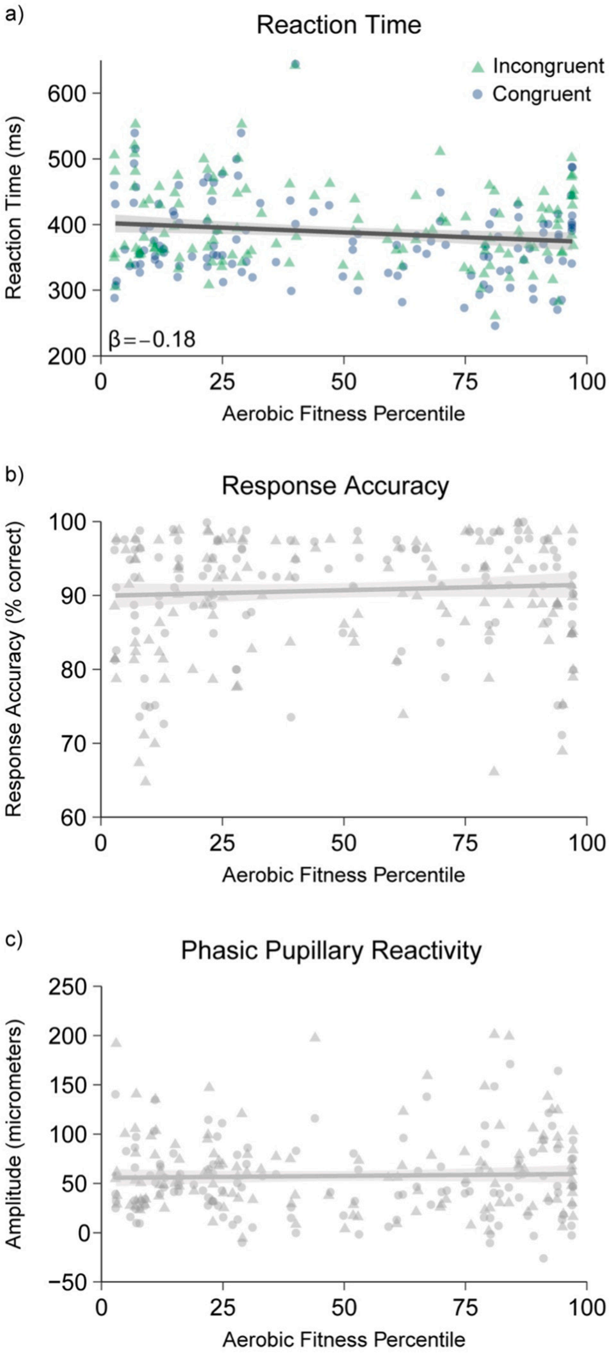

**Fig. 1.** Scatterplots depicting the relationship between aerobic fitness and a) mean reaction time, b) response accuracy, and c) phasic pupillary response. Congruent trials are depicted with a circle. Incongruent trials are depicted with a triangle.

#### 2.1.2. Response accuracy

Analysis indicated that aerobic fitness was unrelated to response accuracy (B = 0.02 [95% CI: −0.02 to 0.05], SE B = 0.02, Beta = 0.08) after accounting for the influence of demographic factors and task congruency, $R^2_{change} < 0.01$, $F_{change}(6, 243) = 1.1$, $p = 0.3$, $f^2 < 0.01$ [95% CI: 0.0 to 0.02] (see Fig. 1b). The inclusion of an interaction term between fitness and congruency did not improve model fit ($R^2_{change} < 0.01$, $p = 0.8$).

#### 2.1.3. Phasic activity of the locus-coeruleus

Replicating the existing pupillometry literature (van der Wel and van Steenbergen, 2018), the difference between phasic pupillary reactivity on congruent (51.9 ± 37.5 μm) and incongruent (63.2 ± 42.5 μm) trials was statistically significant: $t(125) = 4.3$, $p < 0.001$, $d_{rm} = 0.28$ [95% CI: 0.15 to 0.41] (see Fig. 2). However, hierarchical regression analysis indicated that aerobic fitness was unrelated to phasic activity of the locus-coeruleus (B = −0.01 [95% CI: −0.18 to 0.17], SE B = 0.09, Beta = −0.01) after accounting for the influence of demographic factors and task congruency, $R^2_{change} < 0.01$, $F_{change}(6, 243) < 0.1$, $p = 0.9$, $f^2 < 0.01$ [95% CI: 0.0 to 0.01] (see Fig. 1c). The inclusion of an interaction term between fitness and congruency did not improve model fit ($R^2_{change} < 0.01$, $p = 0.9$).

### 2.2. Mediating influence of phasic activity of the locus-coeruleus

Although the initial model failed to observe a relationship between aerobic fitness and phasic activity of the locus-coeruleus (as assessed using task-evoked pupillary reactivity), the a priori analytical plan was to examine the extent to which phasic activity of the locus-coeruleus mediated the relationship between aerobic fitness and behavioral indices of inhibitory control (reaction time and response accuracy). Accordingly, secondary analyses were performed by comparing a series of linear regression models using the “mediation” (version 4.4.7) (Tingley et al., 2014) and “Rmimic” (version 1.0.3) (Pontifex, 2020) R packages with unstandardized indirect effects computed using 1000 quasi-Bayesian approximation based samples.

Analysis observed that the relationship between aerobic fitness and reaction time was not mediated by phasic activity of the locus-coeruleus (Proportion Mediated <0.01%; Average Causal Mediation Effect = −0.0 [95% CI: −0.02 to 0.02], $p > 0.99$; Average Direct Effect = −0.34 [95% CI: −0.61 to −0.06], $p = 0.014$), while accounting for the influence of demographic factors and task congruency. Similarly, the relationship

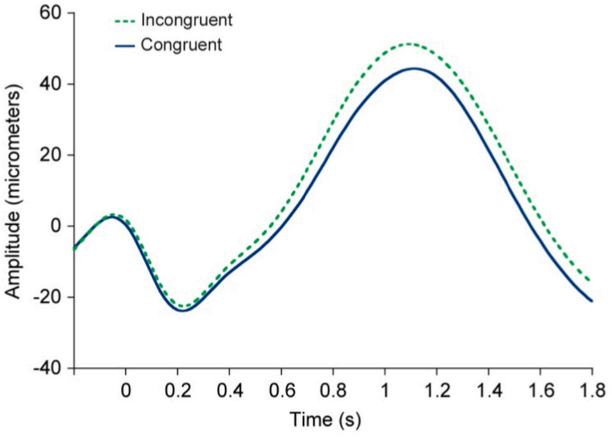

**Fig. 2.** Grand average waveforms showing the difference in pupillary dilation between congruent and incongruent trials of the flanker task.

between aerobic fitness and response accuracy was not mediated by phasic activity of the locus-coeruleus (Proportion Mediated = 0.2%; Average Causal Mediation Effect = −0.0 [95% CI: −0.01 to 0.01], $p >$ 0.99; Average Direct Effect = 0.02 [95% CI: −0.02 to 0.05], $p = 0.3$), while accounting for the influence of demographic factors and task congruency. Of note, the results of the mediation analysis remained the same even when using different baseline approaches (i.e., baseline corrected using the −1000 to 0 ms pre-stimulus window), and different quantification approaches (i.e., mean amplitude and peak interval amplitude).

## 3. Discussion

Replicating a robust body of literature (Alderman and Olson, 2014; Buck et al., 2008; Hillman et al., 2009; Huang et al., 2015; Mora-Gonzalez et al., 2020; Pontifex et al., 2011; Pontifex et al., 2014a; Westfall et al., 2018), aerobic fitness was associated with superior performance on a behavioral assessment of inhibitory aspects of cognitive control. Specifically, greater aerobic fitness was associated with faster responding to the target stimulus of the flanker task of inhibitory control, with no such fitness-related associations for response accuracy. The lack of a relationship for response accuracy is perhaps unsurprising given the use of a high-functioning population and the prolonged inter-trial interval to allow sufficient time for the phasic pupillary response, which served to reduce the variability in response accuracy and potentially mitigated the opportunity for aerobic fitness to exert an influence over this criterion of performance. As only a single task was implemented, the ability of the present investigation to make broad statements regarding a selective relationship between fitness and inhibitory control/cognitive control is tempered — particularly given the finding of only an overall relationship with reaction time. Nevertheless, the overarching aim of the present investigation was to examine the extent to which phasic activation of the locus-coeruleus might mediate (either fully or partially) the association between aerobic fitness and superior performance on inhibitory aspects of cognitive control. Accordingly, the findings of the present investigation replicated this well-established pattern of results to enable examination of phasic activation of the locus-coeruleus as a potential mediator.

Due to the high bioenergetic cost of neurons in the locus-coeruleus – and the difficulty of maintaining high levels of phasic activity in this system over time – it was expected that higher-fit individuals would have had larger pupillary reactivity throughout the task, indicating a higher level of task engagement. Given a robust and consistent body of neuroimaging work indicating higher levels of task engagement, varying as a function of the level of cognitive control required, for higher- relative to lower-aerobically-fit individuals (e.g., Kao et al., 2019; Pontifex et al., 2009, 2012), this hypothesis had considerable empirical support. In addition, higher levels of aerobic fitness are related to increased cerebral blood flow velocity across the lifespan (Ainslie et al., 2008) – and so an increased ability to sustain phasic activity of the locus-coeruleus would certainly make sense in the context of this phenomenon.

Contrary to our a priori hypotheses, however, phasic locus-coeruleus activity was not observed to mediate the relationship between aerobic fitness and performance on inhibitory aspects of cognitive control, nor was phasic locus-coeruleus activity even observed to relate to aerobic fitness. Thus, such findings provide preliminary evidence to suggest that fitness does not alter activity levels of the locus-coeruleus. Despite a relatively robust sample size, the ability to detect small effect sizes, and the use of a well-established assessment of cognition in the present investigation with findings that replicated the extant literature, it is important to note that participants in this investigation were college-aged young adults. As this population is quite high-functioning, it may be that the contribution of differences in locus-coeruleus activity may only be apparent in developing populations, cognitively impaired adults, or other populations with sub-optimal levels of cognitive functioning. Further, while the activity levels of the locus-coeruleus exhibited typical patterns of modulation in response to the greater level of inhibitory control required for the incongruent trials of the flanker task (van der Wel and van Steenbergen, 2018), it may be that a relationship between aerobic fitness and activity levels of the locus-coeruleus may only become apparent in response to tasks with a greater level of difficulty. In addition, the cross-sectional nature of the present investigation does not allow for causal conclusions to be drawn. Thus, further research – especially randomized, controlled trials examining how changes in fitness are related to changes in pupillary reactivity and/or cognitive task performance – is necessary to better characterize the nature of this relationship.

Collectively, despite the lack of a relationship between aerobic fitness and phasic activation of the locus-coeruleus, this work contributes towards a broader understanding of those neural differences that may underlie the manifestation of superior behavioral performance on tests indexing aspects of cognitive control for those with superior levels of aerobic fitness. As the acquisition of the attribute of aerobic fitness is in large part a consequence of the chronic and habitual engagement in physical activity of an aerobic nature, investigating such relationships with regard to aerobic fitness provides insight into the potential benefit of accumulating such physical activity behaviors over the course of many years or even decades. Understanding the mechanisms that underlie superior behavioral performance might therefore enable the development of physical activity interventions optimized to promote and sustain enhancements in cognitive health and function. Accordingly, it is essential that future investigations in this area continue to investigate the potential mechanisms underlying these relationships as well as to what extent these mechanisms respond to changes in physically active behaviors.

## Declaration of competing interest

No conflicting financial interests exist.

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## *CRediT authorship contribution statement*

**Madison C. Chandler**: Formal Analysis; Investigation; Data Curation; Writing – Original Draft; Writing – Review & Editing; Visualization; Project Administration; Funding Acquisition. **Amanda L. McGowan**: Conceptualization; Methodology; Software; Validation; Investigation; Resources; Data Curation; Writing – Review & Editing; Supervision; Funding Acquisition. **Jan W. Brascamp**: Methodology; Validation; Writing – Review & Editing. **Matthew B. Pontifex**: Conceptualization; Methodology; Software; Validation; Formal Analysis; Writing – Original Draft; Writing – Review & Editing; Visualization; Supervision; Project Administration.

## References

Ainslie, P.N., Cotter, J.D., George, K.P., Lucas, S., Murrell, C., Shave, R., Thomas, K.N., Williams, M.J.A., Atkinson, G., 2008. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. J. Physiol. 586 (16), 4005–4010. https://doi.org/10.1113/jphysiol.2008.158279.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19 (6), 716–723.

Alderman, B.L., Olson, R.L., 2014. The relation of aerobic fitness to cognitive control and heart rate variability: a neurovisceral integration study. Biol. Psychol. 99, 26–33. https://doi.org/10.1016/j.biopsycho.2014.02.007.

American College of Sports Medicine, 2018. ACSM's Guidelines for Exercise Testing and Prescription, 10th ed. Lippincott Williams & Wilkins.

Aston-Jones, G., Cohen, J.D., 2005. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu. Rev. Neurosci. 28 (1), 403–450. https://doi.org/10.1146/annurev.neuro.28.061604.135709.

Aston-Jones, G., Rajkowski, J., Kubiak, P., Alexinsky, T., 1994. Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J. Neurosci. 14 (7), 4467–4480.

Bekar, L.K., Wei, H.S., Nedergaard, M., 2012. The locus coeruleus-norepinephrine network optimizes coupling of cerebral blood volume with oxygen demand. J. Cereb. Blood Flow Metab. 32 (12), 2135–2145. https://doi.org/10.1038/jcbfm.2012.115.

Bellesi, M., Tononi, G., Cirelli, C., Serra, P.A., 2016. Region-specific dissociation between cortical noradrenaline levels and the sleep/wake cycle. Sleep 39 (1), 143–154. https://doi.org/10.5665/sleep.5336.

Bouret, S., Sara, S.J., 2005. Network reset: a simplified overarching theory of locus coeruleus noradrenaline function. Trends Neurosci. 28 (11), 574–582. https://doi.org/10.1016/j.tins.2005.09.002.

Brassell, A.A., Shoulberg, E.K., Pontifex, M.B., Smith, A.L., Paoli, A.G.D., Hoza, B., 2015. Aerobic fitness and inhibition in young children: moderating roles of ADHD status and age. J. Clin. Child Adolesc. Psychol. 0 (0), 1–7. https://doi.org/10.1080/15374416.2015.1063431.

Buck, S.M., Hillman, C.H., Castelli, D.M., 2008. The relation of aerobic fitness to Stroop task performance in preadolescent children. Med. Sci. Sports Exerc. 40, 166–172.

Bunce, D., Murden, F., 2006. Age, aerobic fitness, executive function, and episodic memory. Eur. J. Cogn. Psychol. 18 (2), 221–233. https://doi.org/10.1080/09541440540000185.

Carter, M.E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., Deisseroth, K., de Lecea, L., 2010. Tuning arousal with optogenetic modulation of locus coeruleus neurons. Nat. Neurosci. 13 (12), 1526–1533. https://doi.org/10.1038/nn.2682.

Causse, M., Peysakhovich, V., Fabre, E.F., 2016. High working memory load impairs language processing during a simulated piloting task: an ERP and pupillometry study. Front. Hum. Neurosci. 10 https://doi.org/10.3389/fnhum.2016.00240.

Chaddock, L., Erickson, K.I., Prakash, R.S., Voss, M.W., VanPatter, M., Pontifex, M.B., Hillman, C.H., Kramer, A.F., 2012. A functional MRI investigation of the association between childhood aerobic fitness and neurocognitive control. Biol. Psychol. 89 (1), 260–268. https://doi.org/10.1016/j.biopsycho.2011.10.017.

Colcombe, S.J., Kramer, A.F., 2003. Fitness effects on the cognitive function of older adults: a meta-analytic study. Psychol. Sci. 14, 125–130. https://doi.org/10.1111/1467-9280.t01-1-01430.

Colcombe, S.J., Kramer, A.F., Erickson, K.I., Scalf, P., McAuley, E., Cohen, N.J., Webb, A., Jerome, G.J., Marquez, D.X., Elavsky, S., 2004. Cardiovascular fitness, cortical plasticity, and aging. Proc. Natl. Acad. Sci. U. S. A. 101 (9), 3316–3321.

Costa, V.D., Rudebeck, P.H., 2016. More than meets the eye: the relationship between pupil size and locus coeruleus activity. Neuron 89 (1), 8–10. https://doi.org/10.1016/j.neuron.2015.12.031.

de Bilbao, F., Jazat, F., Lamour, Y., Senut, M.C., 1991. Age-related changes in galanin-immunoreactive cells of the rat medial septal area. J. Comp. Neurol. 313 (4), 613–624. https://doi.org/10.1002/cne.903130407.

Delorme, A., Makeig, S., 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics. J. Neurosci. Methods 134, 9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009.

Donnelly, J.E., Hillman, C.H., Castelli, D., Etnier, J.L., Lee, S., Tomporowski, P., Lambourne, K., Szabo-Reed, A.N., 2016. Physical activity, fitness, cognitive function, and academic achievement in children: a systematic review. Med. Sci. Sports Exerc. 48 (6), 1223–1224. https://doi.org/10.1249/MSS.0000000000000966.

Eriksen, C.W., Eriksen, B.A., 1974. Effects of noise letters upon the identification of a target letter in a non-search task. Percept. Psychophys. 25, 249–263. https://doi.org/10.3758/BF03203267.

Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 29, 175–191. https://doi.org/10.3758/BF03193146.

Geva, R., Zivan, M., Warsha, A., Olchik, D., 2013. Alerting, orienting or executive attention networks: differential patters of pupil dilations. Front. Behav. Neurosci. 7 https://doi.org/10.3389/fnbeh.2013.00145.

Gilzenrat, M.S., Nieuwenhuis, S., Jepma, M., Cohen, J.D., 2010. Pupil diameter tracks changes in control state predicted by the adaptive gain theory of locus coeruleus function. Cognitive, Affective & Behavioral Neuroscience 10 (2), 252–269. https://doi.org/10.3758/CABN.10.2.252.

Heitz, R.P., Schrock, J.C., Payne, T.W., Engle, R.W., 2008. Effects of incentive on working memory capacity: behavioral and pupillometric data. Psychophysiology 45 (1), 119–129. https://doi.org/10.1111/j.1469-8986.2007.00605.x.

Hillman, C.H., Buck, S.M., Themanson, J.R., Pontifex, M.B., Castelli, D.M., 2009. Aerobic fitness and cognitive development: event-related brain potential and task performance indices of executive control in preadolescent children. Dev. Psychol. 45 (1), 114–129. https://doi.org/10.1037/a0014437.

Hopstaken, J.F., van der Linden, D., Bakker, A.B., Kompier, M.A.J., 2015. The window of my eyes: task disengagement and mental fatigue covary with pupil dynamics. Biol. Psychol. 110, 100–106. https://doi.org/10.1016/j.biopsycho.2015.06.013.

Huang, T., Tarp, J., Domazet, S.L., Thorsen, A.K., Froberg, K., Andersen, L.B., Bugge, A., 2015. Associations of adiposity and aerobic fitness with executive function and math performance in Danish adolescents. J. Pediatr. 167 (4), 810–815. https://doi.org/10.1016/j.jpeds.2015.07.009.

Joshi, S., Li, Y., Kalwani, R.M., Gold, J.I., 2016. Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. Neuron 89 (1), 221–234. https://doi.org/10.1016/j.neuron.2015.11.028.

Kao, S.-C., Cadenas-Sanchez, C., Shigeta, T.T., Walk, A.M., Chang, Y.-K., Pontifex, M.B., Hillman, C.H., 2019. A systematic review of physical activity and cardiorespiratory fitness on P3b. Psychophysiology e13425.

Kinomura, S., Larsson, J., Gulyás, B., Roland, P.E., 1996. Activation by attention of the human reticular formation and thalamic intralaminar nuclei. Science 271 (5248), 512–515. https://doi.org/10.1126/science.271.5248.512.

Knapen, T., de Gee, J.W., Brascamp, J.W., Nuiten, S., Hoppenbrouwers, S., Theeuwes, J., 2016. Cognitive and ocular factors jointly determine pupil responses under equiluminance. PLoS One 11 (5), e0155574. https://doi.org/10.1371/journal.pone.0155574.

Koenker, R., 1981. A note on studentizing a test for heteroscedasticity. J. Econ. 17 (1), 107–112. https://doi.org/10.1016/0304-4076(81)90062-2.

Laeng, B., Ørbo, M., Holmlund, T., Miozzo, M., 2011. Pupillary Stroop effects. Cogn. Process. 12 (1), 13–21. https://doi.org/10.1007/s10339-010-0370-z.

Luque-Casado, A., Perakakis, P., Hillman, C.H., Kao, S.-C., Llorens, F., Guerra, P.A.M., Sanabria, D., 2016. Differences in sustained attention capacity as a function of aerobic fitness. Med. Sci. Sports Exerc. 48 (5), 887–895. https://doi.org/10.1249/MSS.0000000000000857.

Mather, M., Harley, C.W., 2016. The locus coeruleus: essential for maintaining cognitive function and the aging brain. Trends Cogn. Sci. 20 (3), 214–226. https://doi.org/10.1016/j.tics.2016.01.001.

McGowan, A.L., Chandler, M.C., Brascamp, J.W., Pontifex, M.B., 2019. Pupillometric indices of locus-coeruleus activation are not modulated following single bouts of exercise. Int. J. Psychophysiol. 140, 41–52. https://doi.org/10.1016/j.ijpsycho.2019.04.004.

Meyer, D.E., Kieras, D.E., 1997. A computational theory of executive cognitive processes and multiple-task performance: part 1. Basic mechanisms. Psychol. Rev. 104, 3–65.

Mora-Gonzalez, J., Esteban-Cornejo, I., Solis-Urra, P., Migueles, J.H., Cadenas-Sanchez, C., Molina-Garcia, P., Rodriguez-Ayllon, M., Hillman, C.H., Catena, A., Pontifex, M.B., Ortega, F.B., 2020. Fitness, physical activity, sedentary time, inhibitory control, and neuroelectric activity in children with overweight or obesity: the ActiveBrains project. Psychophysiology 57 (6), e13579. https://doi.org/10.1111/psyp.13579.

Murphy, P.R., Robertson, I.H., Balsters, J.H., O'Connell, R.G., 2011. Pupillometry and P3 index the locus coeruleus–noradrenergic arousal function in humans. Psychophysiology 48 (11), 1532–1543. https://doi.org/10.1111/j.1469-8986.2011.01226.x.

Murphy, P.R., Vandekerckhove, J., Nieuwenhuis, S., 2014. Pupil-linked arousal determines variability in perceptual decision making. PLoS Comput. Biol. 10 (9), e1003854.

Murray, P.S., Groves, J.L., Pettett, B.J., Britton, S.L., Koch, L.G., Dishman, R.K., Holmes, P.V., 2010. Locus coeruleus galanin expression is enhanced after exercise in rats selectively bred for high capacity for aerobic activity. Peptides 31 (12), 2264–2268. https://doi.org/10.1016/j.peptides.2010.09.005.

Norman, D.A., Shallice, T., 1986. Attention to action: willed and automatic control of behavior. In: Consciousness and Self-regulation: Advances in Research and Theory, vol. 4. Plenum, pp. 1–18.

Peirce, J.W., 2009. Generating stimuli for neuroscience using PsychoPy. Frontiers in Neuroinformatics 2, 10. https://doi.org/10.3389/neuro.11.010.2008.

Pfeiffer, K.A., Pivarnik, J.M., Womack, C.J., Reeves, M.J., Malina, R.M., 2002. Reliability and validity of the Borg and OMNI rating of perceived exertion scales in adolescent girls. Med. Sci. Sports Exerc. 34 (12), 2057–2061. https://doi.org/10.1097/00005768-200212000-00029.

Pontifex, M.B., 2020. Rmimic: an R package that mimic outputs of popular commercial statistics software packages with effect sizes and confidence intervals. (1.0) [computer software]. https://github.com/mattpontifex/Rmimic.

Pontifex, M.B., Hillman, C.H., Polich, J., 2009. Age, physical fitness, and attention: P3a and P3b. Psychophysiology 46 (2), 379–387. https://doi.org/10.1111/j.1469-8986.2008.00782.x.

Pontifex, M.B., Raine, L.B., Johnson, C.R., Chaddock, L., Voss, M.W., Cohen, N.J., Kramer, A.F., Hillman, C.H., 2011. Cardiorespiratory fitness and the flexible modulation of cognitive control in preadolescent children. J. Cogn. Neurosci. 23 (6), 1332–1345. https://doi.org/10.1162/jocn.2010.21528.

Pontifex, M.B., Scudder, M.R., Drollette, E.S., Hillman, C.H., 2012. Fit and vigilant: the relationship between poorer aerobic fitness and failures in sustained attention during preadolescence. Neuropsychology 26 (4), 407–413. https://doi.org/10.1037/a0028795.

Pontifex, M.B., Kamijo, K., Scudder, M.R., Raine, L.B., Khan, N.A., Hemrick, B., Evans, E. M., Castelli, D.M., Frank, K.A., Hillman, C.H., 2014a. The differential association of adiposity and fitness with cognitive control in preadolescent children. Monogr. Soc. Res. Child Dev. 79 (4), 72–92. https://doi.org/10.1111/mono.12131.

Pontifex, M.B., Parks, A.C., O'Neil, P.C., Egner, A.R., Warning, J.T., Pfeiffer, K.A., Fenn, K.M., 2014b. Poorer aerobic fitness relates to reduced integrity of multiple memory systems. Cognitive, Affective, & Behavioral Neuroscience 14 (3), 1132–1141. https://doi.org/10.3758/s13415-014-0265-z.

Pontifex, M.B., Gwizdala, K.L., Parks, A.C., Pfeiffer, K.A., Fenn, K.M., 2016. The association between physical activity during the day and long-term memory stability. Sci. Rep. 6, 38148 https://doi.org/10.1038/srep38148.

R Core Team, 2019. R: a language and environment for statistical computing (3.6.1) [computer software]. https://www.R-project.org/.

Raine, L.B., Lee, H.K., Saliba, B.J., Chaddock-Heyman, L., Hillman, C.H., Kramer, A.F., 2013. The influence of childhood aerobic fitness on learning and memory. PLoS One 8 (9), e72666. https://doi.org/10.1371/journal.pone.0072666.

Sara, S.J., Bouret, S., 2012. Orienting and reorienting: the locus coeruleus mediates cognition through arousal. Neuron 76 (1), 130–141. https://doi.org/10.1016/j.neuron.2012.09.011.

Scharinger, C., Soutschek, A., Schubert, T., Gerjets, P., 2015. When flanker meets the n-back: what EEG and pupil dilation data reveal about the interplay between the two central-executive working memory functions inhibition and updating. Psychophysiology 52 (10), 1293–1304. https://doi.org/10.1111/psyp.12500.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52 (3–4), 591–611. https://doi.org/10.1093/biomet/52.3-4.591.

Shvartz, E., Reibold, R.C., 1990. Aerobic fitness norms for males and females aged 6 to 75 years: a review. Aviat. Space Environ. Med. 61, 3–11.

Themanson, J.R., Pontifex, M.B., Hillman, C.H., 2008. Fitness and action monitoring: evidence for improved cognitive flexibility in young adults. Neuroscience 157 (2), 319–328. https://doi.org/10.1016/j.neuroscience.2008.09.014.

Thomas, S., Reading, J., Shephard, R.J., 1992. Revision of the physical activity readiness questionnaire (PAR-Q). Canadian Journal of Sport Sciences 17, 338–345.

Tingley, D., Yamamoto, T., Hirose, K., Keele, L., Imai, K., 2014. Mediation: R package for causal mediation analysis. J. Stat. Softw. 59 (5) https://doi.org/10.18637/jss.v059.i05.

van der Wel, P., van Steenbergen, H., 2018. Pupil dilation as an index of effort in cognitive control tasks: a review. Psychon. Bull. Rev. 25 (6), 2005–2015. https://doi.org/10.3758/s13423-018-1432-y.

van Steenbergen, H., Band, G.P.H., 2013. Pupil dilation in the Simon task as a marker of conflict processing. Front. Hum. Neurosci. 7 https://doi.org/10.3389/fnhum.2013.00215.

Voss, M.W., Chaddock, L., Kim, J.S., VanPatter, M., Pontifex, M.B., Raine, L.B., Cohen, N. J., Hillman, C.H., Kramer, A.F., 2011. Aerobic fitness is associated with greater efficiency of the network underlying cognitive control in preadolescent children. Neuroscience 199, 166–176. https://doi.org/10.1016/j.neuroscience.2011.10.009.

Westfall, D.R., Kao, S.-C., Scudder, M.R., Pontifex, M.B., Hillman, C.H., 2017. The association between aerobic fitness and congruency sequence effects in preadolescent children. Brain Cogn. 113, 85–92. https://doi.org/10.1016/j.bandc.2016.12.005.

Westfall, D.R., Gejl, A.K., Tarp, J., Wedderkopp, N., Kramer, A.F., Hillman, C.H., Bugge, A., 2018. Associations between aerobic fitness and cognitive control in adolescents. Front. Psychol. 9 https://doi.org/10.3389/fpsyg.2018.01298.

Wrenn, C.C., Crawley, J.N., 2001. Pharmacological evidence supporting a role for galanin in cognition and affect. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 25 (1), 283–299. https://doi.org/10.1016/S0278-5846(00)00156-1.