Tyrosine negatively affects flexible-like behaviour under cognitively demanding conditions

ROBSON, Anna, LIM, Lee Wei and AQUILI, Luca <http://orcid.org/0000-0003-4930-1536>

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Tyrosine negatively affects flexible-like behaviour under cognitively demanding conditions

Anna Robson[1*], Lee Wei Lim[2], Luca Aquili[1]

[1]Department of Psychology, Sociology and Politics, Sheffield Hallam University, Sheffield, UK

[2]School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, SAR, China

* Corresponding author

E-mail: ar2091@exchange.shu.ac.uk

Postal Address: Anna Robson, Heart of the Campus, Department of Psychology, Sociology and Politics, Collegiate Campus, Collegiate Crescent, Sheffield, S10 2BP

Background: The catecholaminergic precursor to dopamine, tyrosine, is an important modulator of cognitive performance. A number of studies have demonstrated that the beneficial effects of tyrosine on cognitive performance are most pronounced when individuals are exposed to stressful situations, such as hypothermia. However, little is known about whether manipulation of stress using non-aversive stimuli, such as cognitive demand, can also bring about similar improvements.

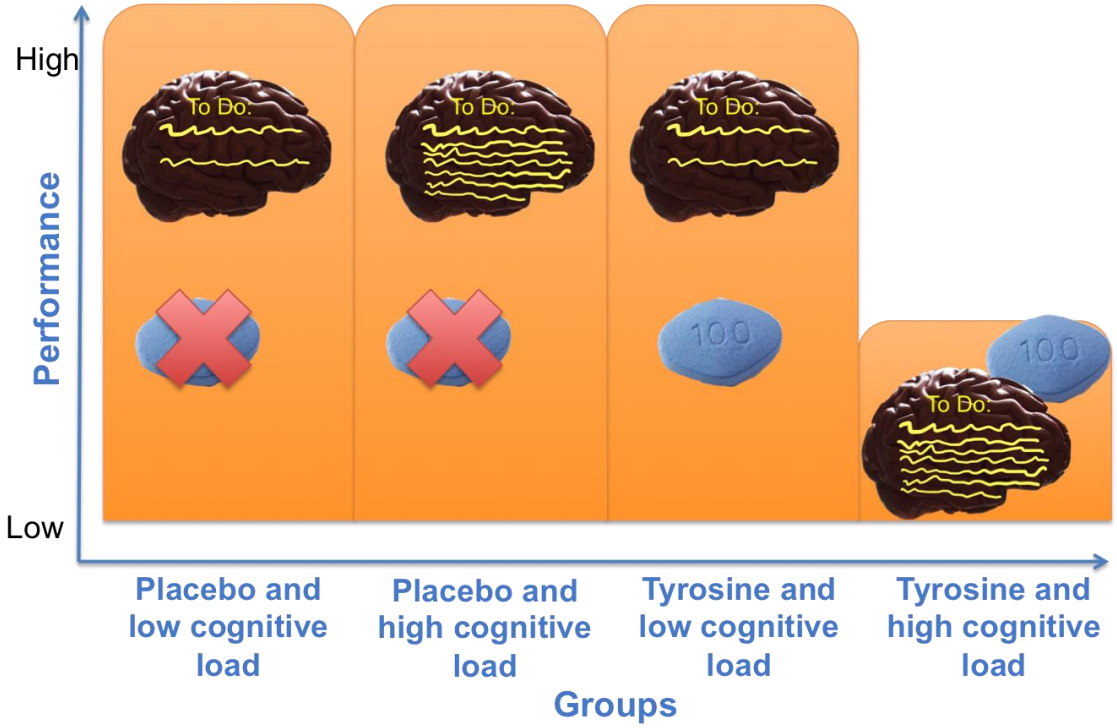
Methods: We conducted a randomized, double-blind, placebo-controlled experiment to test the effects of tyrosine administration and cognitive load (low or high) on cognitive flexibility, a measure known to be influenced by catecholaminergic function. A total of 70 healthy volunteers completed a baseline cognitive flexibility test (Wisconsin Card Sorting Test: WCST). Participants were given a dose of either tyrosine (2.0 g) or placebo (cellulose) and subject to either low cognitive load (simple reaction time task) or high cognitive load (digit memory span task), immediately followed by a WCST for a second time.

Results: Contrary to expectations, we found that instead of ameliorating performance under the high cognitive load condition, tyrosine worsened cognitive flexibility.

Limitations: Physiological marker of stress was not measured.

Conclusions: Our results suggest that aversive stressors and cognitive demand modulate the effects of tyrosine on cognitive performance in a differential manner.

Keywords: Tyrosine, Dopamine; Cognitive flexibility



The effect of Tyrosine and cognitive load on cognitive flexibility shown in a graphical abstract.

Introduction

Cognitive flexibility is the brain's ability to think about multiple concepts at the same time and quickly switch between concepts (Majdic et al., 2017), which can be tested using various paradigms including reversal learning and set shifting. The neuronal circuitry underpinning cognitive flexibility encompasses parts of the prefrontal cortex and the striatum, and the catecholaminergic neurotransmitter dopamine acts as an important modulator of fronto-striatal activity (Klanker, Feenstra & Denys, 2013).

Studies involving the pharmacological manipulation of the dopaminergic system have revealed that increased dopaminergic transmission through D_2 receptors was beneficial to set shifting performance (Van Holstein et al., 2011) but not to reversal learning

(Cools et al., 2009). A similar finding was reported when the dopamine precursor L-Dopa was administered to patients with Parkinson's disease, which improved set shifting but impaired reversal learning performance (Cools, 2006). Studies on amphetamine as a psychostimulant have reinforced the idea that dopaminergic activity and cognitive performance have an inverted U-shaped relationship (Cools and D'Esposito, 2011), with low or high doses impairing reversal learning (Idris, Repeto & Neill, 2005) but intermediate doses leaving performance intact (Soto et al., 2012).

More recently, a number of studies have investigated the potential effect of the dopaminergic precursor tyrosine on cognitive flexibility, which theoretically might offer a number of advantages over L-Dopa. Unlike L-Dopa, the conversion of tyrosine to dopamine is restricted by competition from other endogenous amino acids and by the rate-limiting tyrosine-hydroxylase enzyme (Jongkees, Hommel, Kühn & Colzato, 2015). These restrictions comparatively limit the overall enhancement of dopamine levels by tyrosine, and reduce the likelihood of shifting participants to the far end of the inverted U-shaped curve.

Tyrosine administration has been shown to improve task switching (Steenbergen, Sellaro, Hommel & Colzato, 2015). Our group found tyrosine had beneficial effects on set shifting, which was dependent on dorsolateral prefrontal cortex activity (Dennison, Gao, Lim, Stagg & Aquili, 2019). However, reports on the effectiveness of tyrosine on cognition are rather more inconsistent (Jongkees et al., 2015). Some of this heterogeneity is related to the clinical population tested (e.g. depression vs ADHD) (Gelenberg et al., 1990; Posner et al., 2009), and due to inter-individual differences of dopaminergic gene expression in the striatum (Colzato et al., 2016). Moreover, it has

been suggested that the positive cognitive effects of tyrosine may be most prominent when individuals are exposed to stressful situations (Jongkees et al., 2015).

Aversive stimuli such as stress increase catecholamine activity and use up resources, resulting in the depletion of neurotransmitter levels and behavioural depression (Kvetnansky, Sabban & Palkovits, 2009). Under these circumstances, tyrosine can act to replenish this depletion. In studies on hypothermia as the stressor, tyrosine administration reversed the impairments on attention and memory (Mahoney, Castellani, Kramer, Young & Lieberman, 2007). Additional stressors in which tyrosine has been shown to have beneficial effects include sleep deprivation and an auditory stressor (Deijen and Orlebeck, 1994; Magill et al., 2003). Non-aversive stimuli such as high cognitive demand have also been hypothesized to lead to similar catecholaminergic depletion (Jongkees et al., 2015), but this has been hardly investigated. Thomas, Lockwood, Singh & Deuster (1999) were the first to show that tyrosine improved working memory performance only when performing multiple tasks simultaneously. Finding out whether tyrosine has enhancing effects only under particularly challenging conditions such as high cognitive load would be important as it would confirm that catecholaminergic depletion can be reversed both when individuals are exposed to overt and non-overt stressors.

Method

We conducted a randomized, double-blind, placebo-controlled study to test whether tyrosine beneficial effects on cognition during aversive stressful conditions (e.g., hypothermia) could be recreated using a non-aversive stressful stimuli (e.g., high cognitive load). In addition, we wanted to test the effect on a different domain,

cognitive flexibility, as tyrosine administration was shown to ameliorate cognitive flexibility performance under normal conditions (Steenbergen et al., 2015; Dennison et al., 2019).

This study was approved by the ethics committee of Sheffield Hallam University and was conducted in compliance with the Declaration of Helsinki (World Medical Association, 1964). Participants consisted of 70 university students (M=19.9 years, SD=1.6) including 59 females and 11 males). Written informed consent was obtained from all participants in the study**.** Exclusion criteria included individuals with cardiac, hepatic, renal and neurological disorders, history of alcohol or drug addiction, and psychiatric illness, as well as those with a history of taking tyrosine supplements.

Participants were randomly assigned to the tyrosine or placebo groups. Participants received either 2.0 g of tyrosine (BulkPowders Ltd, UK.) or 2.0 g of microcrystalline cellulose (Redwells Creative Limited, UK) dissolved in 400 mL of orange juice as in previously published protocols (Dennison et al., 2019). All participants were tested in the morning (9am-11am) and were asked to refrain from eating or drinking for at least three hours. This is to prevent tyrosine competition with other amino acids which may prevent its effectiveness. Participants waited 60 min before testing, as a previous studyon tyrosine modulation of cognitive flexibility found that the peak plasma concentration level occurred at 60 min following oral administration (Steenbergen et al., 2015)[10].

To assess cognitive flexibility, we used an adapted Wisconsin Card Sorting Test (WCST) implemented in PEBL software (Mueller and Piper, 2014). The WCST provides a measure of task switching behaviour, in which subjects are required to match

a sample card to a set of four reference cards based on one of the following three rules: colour, shape, and number. Following a series of correct matches, the classification rules are changed unexpectedly and the subject must learn to switch responses (Monchi, Petrides, Petre, Worsley & Dagher, 2001). We measured reaction times and perseverative errors. Reaction times reflect the time taken to make a choice following the presentation of the sample and reference cards. Perseverative errors are counted as choosing the same incorrect response following a rule shift (e.g., classification rule shift: shape-colour; perseverative error: shape-shape; non-perseverative error: shape-number). The task lasted between 5 and 7 minutes.

For the cognitive load, participants were asked to complete either a simple reaction time task (low cognitive load) or a forward digit span memory task (high cognitive load) implemented in PEBL software. In the simple reaction time task, participants pressed the space bar as soon as possible following the presentation of a stimulus (the letter "x") in the middle of the screen. The dependent measure of interest was the time taken (reaction time in milliseconds) to respond to the stimulus. In the forward digit span memory task, participants were shown a sequence of digits on the screen, one at a time, starting with a list of three items. Participants were then asked to recall (by typing) the sequence in the exact order as it appeared. Participants had to recall correctly two out of three lists with the same number of items before moving to a list containing additional digits. The dependent measure of interest was the length of the longest list.

After screening for eligibility, participants were instructed to refrain from eating/drinking for a minimum of 3 h to reduce competition from other amino acids that share the same transporter (Fernstrom, 1990). Participants were then required to attend a

session lasting approximately 75 min. They first signed a consent form and then completed a WCST (time 1). They then received either tyrosine or placebo according to the group allocation. After 60 min following tyrosine or placebo intake, half the participants completed a simple reaction time task (low cognitive load), and the other half completed a forward digit span memory task (high cognitive load). As soon as they finished the tasks (approximately 5 min), a WCST was administered for the second time (time 2). Finally, participants were asked to fill out a tyrosine/placebo double-blind questionnaire before being debriefed. An outline of the experimental procedure is shown in Figure 1.

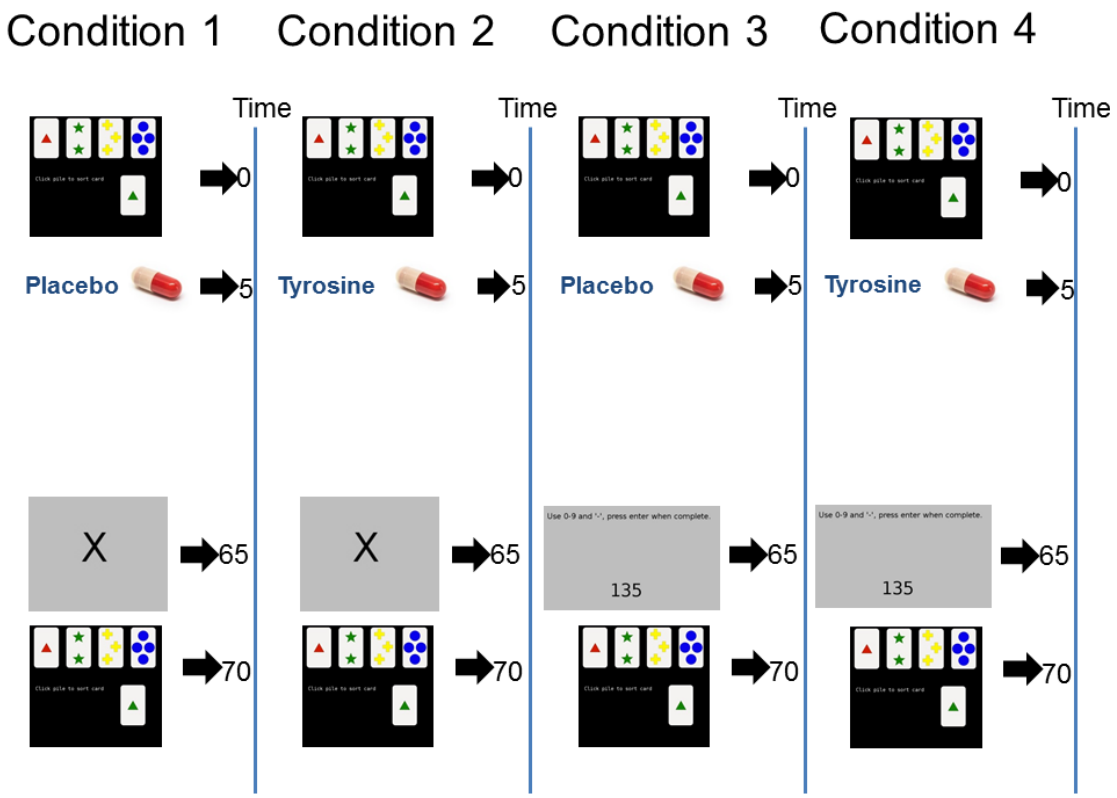


Fig. 1. Graphical illustration of the experimental procedure.

At time 0, all participants completed a WCST as a baseline measure of cognitive flexibility. Approximately 5 min later after completing the WCST, participants were administered either a placebo or tyrosine. After 65 min, which is the time for tyrosine to reach peak concentration in plasma, participants completed either the simple reaction time task (low cognitive load: condition 1 and 2) or the forward digit span memory task (condition 3 and 4). Finally, all participants completed a WCST for the second time.

Results

Statistical analyses were performed using SPSS version 24 (SPSS Inc). The sample size was calculated to achieve a power at 0.8, an alpha level set at 0.05, and a large effect size (η^2) of 0.14 (G*Power 3.1.9.2, Germany). For the two dependent measures of the WCST (reaction time and perseverative errors), we ran a 2x2 factorial ANOVA with drug as one factor (placebo, tyrosine) and cognitive load as the second factor (low and high). Performance of the low and high cognitive load tasks was analysed using an independent samples t-test comparing placebo to tyrosine participants.

The double-blind efficacy of tyrosine/placebo was analysed using a percent correct measure. A score of 100 was given if a participant correctly identified the condition, else a score of 0 was given. A Chi-Square test was used to assess the blinding efficacy.

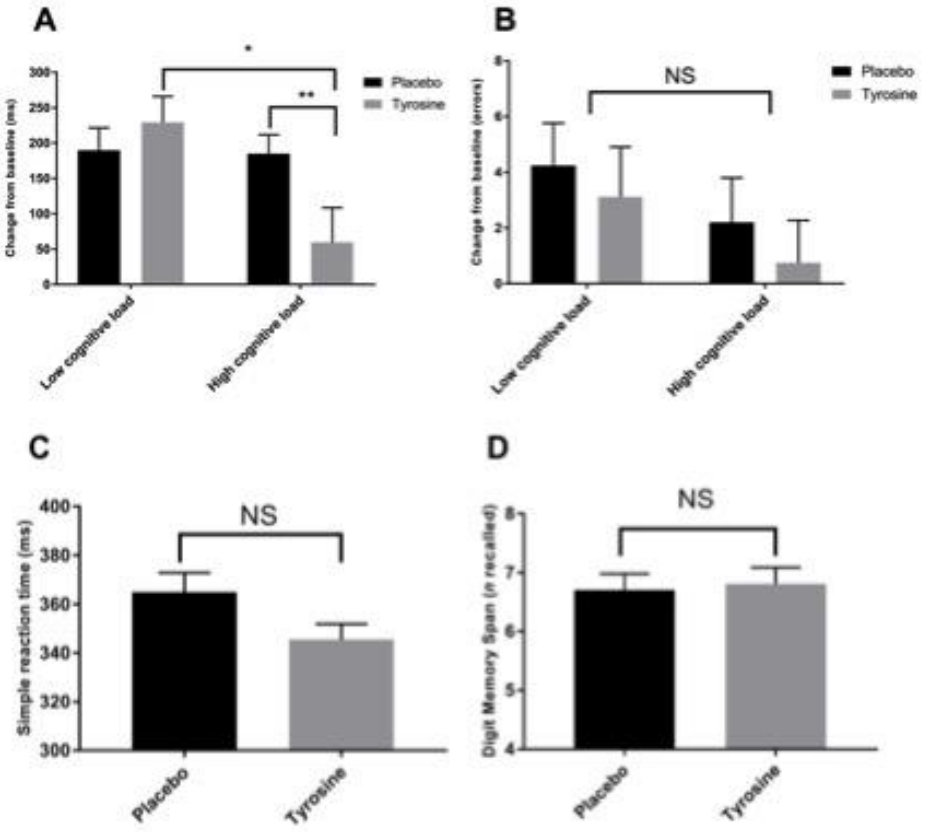
We first analysed changes in reaction times (RT) across conditions. We calculated the change in performance from baseline (Time 1: T1) to post-drug (Time 2: T2) (i.e. [T1] - [T2]), as in a recently published paper (Dennison et al., 2019). A 2x2 factorial between-subjects ANOVA with drug as one factor (Placebo, Tyrosine) and cognitive load as the other factor (Low, High) demonstrated there was no main effect of drug [$F(1, 66) = 1.41$, $p = 0.239$, $\eta^2 = .02$]. However, there was a significant main effect of cognitive load [F (1,

66) =5.84, p=0.018, η^2=.08], with the high cognitive load condition reducing the improvements in reaction times from baseline (M=129.3, SD=168.1) compared to the low cognitive load condition (M=209.5, SD=140.8), which showed greater improvements. Importantly, there was a significant interaction effect between drug and cognitive load [F (1, 66) =5.22, p=0.026, η^2=.07]. To break down this interaction, follow-up simple main effect analyses were performed. For low cognitive load, there was no significant reaction time difference between placebo and tyrosine [F (1, 66) =0.58, p=0.446, η^2=.00], whereas for high cognitive load, tyrosine reduced the improvement in reaction times from baseline compared to placebo [F (1, 66) =6.71, p=0.016, η^2=.08]. Comparing within cognitive loads, there was no significant difference between low and high cognitive loads in placebo participants [F (1, 66) =.00, p=0.924, η^2=.00]. Interestingly, there was a significant difference between low and high cognitive loads in tyrosine participants [F (1, 66) =10.48, p=0.002, η^2=.13], with high cognitive load slowing down reaction times compared to the low condition (Fig 1A).

We next investigated the second measure of cognitive flexibility using the perseverative error, analysed as above. There was no main effect of drug [F (1, 66) =.78, p=0.433, η^2=.00], or main effect of cognitive load [F (1, 66) =1.83, p=0.180, η^2=.02], or significant drug x cognitive load interaction [F (1, 66] =.01, p=0.919, η^2=.00] (Fig 1B).

To ensure the effects of tyrosine on cognitive flexibility were not influenced by changes in simple reaction times (i.e., low cognitive load task) or memory (i.e., high cognitive load task), we ran two independent sample t-tests. There were no significant differences in the performance between placebo and tyrosine participants on the simple reaction time task (t (32) = 1.92, p=0.065) or on the digit span memory task (t (32) = -.28,

210 *p*=0.781). These results confirmed the specificity of the tyrosine effects on cognitive
211 flexibility as modulated by cognitive load (See Fig 2C and 2D).



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213 Fig. 2. A. Effect of drug (Placebo, Tyrosine) and cognitive load (Low, High) on
214 cognitive flexibility as measured by a change in reaction times from baseline (pre-drug).
215 B. Effect of drug (Placebo, Tyrosine) and cognitive load (Low, High) on cognitive
216 flexibility as measured by a change in perseverative errors from baseline (pre-drug). C.
217 Differences in performance between placebo and tyrosine participants on the low
218 cognitive load task (simple reaction time). D. Differences in performance between
219 placebo and tyrosine participants on the high cognitive load task (digit memory span

task). Indication: Error bars represent SEM. * indicates significance at $p<.05$; ** at $p<.01$. NS= not significant when $p>.05$.

The double-blind efficacy of placebo/tyrosine administration was analysed using a Chi-Square test. There was no significant association between the condition (i.e., placebo or tyrosine) and the participant correctly identified it [$_x^2$ (1) = 1.22, p=0.269].

Conclusion

This study aimed to test whether the beneficial effects of tyrosine on cognitive performance under aversive stressful conditions (e.g., hypothermia) as reported in the literature could be replicated under non-aversive but potentially stressful conditions (i.e., cognitive demand). We were particularly interested in measuring cognitive flexibility performance, as this has been shown to have a dopaminergic component (Klanker, Feenstra & Denys, 2013). Contrary to expected results, high cognitive load reduced tyrosine improvements in baseline reaction times when compared to placebo controls. Moreover, the high cognitive load did not produce a performance deficit (compared to the low cognitive load) in the placebo participants, but the opposite was true for those given tyrosine. Significantly, the detrimental effects of tyrosine on cognitive flexibility driven by the high cognitive load manipulation was specific, as tyrosine did not alter performance of the simple reaction time task (low cognitive load) or the forward digit span memory task (high cognitive load).

Previous research using cognitive demand as a non-aversive stressor showed tyrosine had a beneficial effect on memory performance (Thomas et al., 1999). However, tyrosine improved working memory only when multitasking (i.e., high cognitive demand) and not during a simple task battery (i.e., low cognitive demand), which

suggests cognitive demand could induce a stress-like state similar to that elicited by an overt stressor such as hypothermia, and that tyrosine could act to replenish the catecholaminergic depletion (Jongkees et al., 2015). Interestingly, cold exposure as a stressor changed cortisol levels (Mahoney et al., 2007), but the high cognitive demand task did not alter cortisol, indicating cognitive demand may not trigger a physiological stress response. Future studies employing cognitive demand as a proxy for a stressful stimulus would need to further clarify the impact on catecholamine secretion and cortisol.

There are a number of important differences between the current study and that of Thomas et al. (1999), which need to be noted when making comparisons. First, in the study by Thomas et al., they measured working memory, whereas we assessed cognitive flexibility. Although both working memory and cognitive flexibility performance are modulated by the dopaminergic system, several lines of evidence suggest that working memory is primarily mediated by D1 receptors, whilst cognitive flexibility by D2 (Ott and Nieder, 2019). Second, the cognitive load manipulation in the previous study included the simultaneous performance of a number of tasks, whereas we administered either a forward digit memory span or a simple reaction time task. Nevertheless, the high cognitive load task used in this study produced the intended overall (i.e., main effect) detrimental effect on performance. Third, the majority of our participants were females (59/70), and there have been reports of gender differences in response to stress (Allen, Bocek & Burch, 2011) and cognitive flexibility (Kalia et al., 2018).

Previous studies have shown that tyrosine can improve cognitive flexibility under normal, non-stressful conditions (Steenbergen et al, 2015; Dennison et al., 2019).

Although the different types of cognitive flexibility tasks used in these studies and in the present study may provide a partial explanation for the contrasting results, it is still plausible that the beneficial effects of tyrosine on cognitive flexibility could be nullified by the simple attention task and worsened by the more demanding memory test, as demonstrated in our study. Regardless, the precise biological mechanism by which this behavioural effect is mediated needs to be further explored. Furthermore, the finding by Hensel et al. (2019) that showed tyrosine intake caused brain connectivity alterations between the prefrontal cortex and the striatum also needs further investigation.

Limitations: One of the limitations of the study was that we cannot confirm that the high cognitive load task resulted in a physiological stress response as reported in studies using hypothermia as a stressor. The second limitation relates to the gender imbalance in our sample (more females) which only provides partial generalizability of our results.

In conclusion, we provide evidence that high cognitive demand and aversive stressful stimuli (e.g., cold exposure) may have contrasting bidirectional influence on tyrosine administration on cognitive performance.

AR performed the statistical analysis of the data and wrote the manuscript. LWL wrote the manuscript. LA designed the experiments, collected the data, performed the statistical analysis of the data, and wrote the manuscript.

Conflicts of interest: none.

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