SHORT COMMUNICATION

Acute Effect of Cola and Caffeine on Locomotor Activity in Drosophila and Rat

Katarína KMEŤOVÁ[1], Martin MARÔNEK[1], Veronika BORBÉLYOVÁ[1], Július HODOSY[1,2,3], Peter CELEC[1,4,5]

[1]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [2]Emergency Department, University Hospital Bratislava, Slovakia, [3]Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [4]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [5]Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Received December 10, 2020
Accepted February 2, 2021

Summary

Caffeine is well known for reducing fatigue and its effect on behavior is widely studied. Usually, caffeine is not ingested in its pure form but rather in sugar-sweetened beverages such as cola. Our aim was to compare the acute effect of cola and caffeine on locomotor activity. Rats and flies ingested cola or caffeine solution for 24 hours. The open field test revealed higher locomotor activity in cola groups for both flies and rats. Surprisingly, no differences have been observed between caffeine and control group. We conclude that caffeine itself does not explain the effect of cola on locomotor activity. Effect of cola cannot be generalized and interpreted for any caffeinated drink with other contents. Rather, the observed effect on locomotor activity may be caused by interaction of caffeine with other substances present in cola.

Key words

Methylxanthine • Rodent • Sugar-sweetened beverage • Adenosine receptors

Corresponding author

P. Celec, Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia. E-mail: petercelec@gmail.com

Caffeine is the most commonly consumed excitatory substance. Since caffeine has a similar structure to adenine, it acts as an antagonist of the adenosine receptor which delays fatigue and drowsiness (Huang *et al.* 2011). There are multiple studies which described effects of caffeine on different aspects of behavior, such as anxiety (Botton *et al.* 2017), locomotor activity (Bădescu *et al.* 2016, Suh *et al.* 2017), memory (Xu and Reichelt 2018) or circadian rhythm (Suh *et al.* 2017). Acute caffeine application (15 mg/kg) increases locomotor activity in rats (Ettarh *et al.* 2000). However, chronic administration was found to have the opposite effect (Ibrahim *et al.* 2020). Also, Lin et al. showed that caffeine increases locomotor activity in flies, while simultaneously decreasing the length of sleep (Lin *et al.* 2010). The effect of caffeine on anxiety-like behavior is similarly unclear, mainly due to controversial results indicating either anxiogenic (Hughes and Hancock 2017) or even anxiolytic effect (Hughes *et al.* 2014). This means that the effect of caffeine may likely be sensitive to periodicity of application and dose.

Although caffeine is a widely studied substance, it is not usually consumed in its pure form. Presumably, the interpretation of the effects of caffeine as the effects of beverages containing caffeine such as coffee, energy drinks, tea or cola, is a common bias. These caffeinated and often also sugar-sweetened beverages are very popular drinks (Kang and Kim 2017). There are only a handful of studies, studying behavioral effects of cola. Previously, we showed that long term use of cola leads to increased locomotor activity in rats (Celec and Behuliak

PHYSIOLOGICAL RESEARCH • ISSN 1802-9973 (online)

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2010). The rate of locomotor activity could be also observed through changes of body weight. In some studies, weight loss after chronic cola ingestion was observed (Celec 2012, Choi *et al.* 2017). Higher caffeine intake by drinking cola can increase locomotor activity, thus increasing the daily energy expenditure and subsequently weight loss in spite of increased total caloric intake (Celec and Behuliak 2010).

Although the excitatory effect of caffeine has already been studied to some extent using both fruit flies (Keebaugh *et al.* 2017, Ko *et al.* 2017, Nall *et al.* 2016) and rats (Bădescu *et al.* 2016, Celec *et al.* 2010, Celec and Behuliak 2010), studies which directly compare the invertebrate and vertebrate model of caffeine ingestion are rather lacking. Indeed, flies and rats are physiologically different in circadian rhythm and sleep patterns which also affects their baseline locomotor activity. Sleep in flies is defined as behavioral quiescence for 5 minutes or longer (Cirelli and Bushey 2008). The criterion of behavioral quiescence is, however, not enough to distinguish the sleep-like state from quiet wakefulness. During sleep, the organism shows a decreased ability to respond to the external environment. This decrease in activity was observed in flies and, more importantly, a higher arousal threshold during sleep was detected (Hendricks *et al.* 2000, Shaw *et al.* 2000). Similarly to humans, however, flies are diurnal animals with a higher degree of locomotor activity throughout the day and less activity during the night. This is in contrast to rats which are nocturnal animals, although in experimental conditions, it is possible to change their light/dark cycle. Therefore, these two models present different advantages. While drosophila is very accessible to many researchers due to relatively low complexity and a short reproduction cycle, on the other hand, rodents bear a higher degree of physiological similarity compared to humans which makes data extrapolation easier and more accurate. Nevertheless, circadian rhythm is a ubiquitous feature in many organisms across the animal kingdom including drosophila and rat and the fatigue-alleviating effect of caffeine is associated with this mechanism (Ruby *et al.* 2018).

Despite large-scale caffeine market and daily worldwide consumption, studies elucidating the effects of cola on behavior are still lacking. Therefore, we aimed to compare the effect of cola and caffeine on locomotor activity in both invertebrate and vertebrate models represented by flies and rats.

All procedures and animal experiments have been conducted in accordance with Slovak legislation and were approved by the Ethics committee of the Institute of Pathophysiology, Comenius University in Bratislava, Slovakia.

Fly husbandry and medium preparation. A wild type *Drosophila melanogaster* Oregon R strain was used. It was gifted from the laboratory of Dr. Lucia Mentelová (Department of Genetics, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia). Female flies were housed in plastic vials with 5 ml of standard semolina medium (5 g sucrose, 5 g semolina, 2 g dry yeast, 0.8 g agar, and 0.2 g methylparaben per 450 ml of tap water) at 23-25 °C and 12/12 light/dark cycle. Caffeine medium was prepared by adding 35 mg of caffeine into the standard medium before cooking. This amount is equivalent to the amount present in approximately 450 ml of cola. Cola medium was made by adding 450 ml of Coca-Cola instead of water to the standard medium. Standard semolina medium served as control.

Rat housing. Twenty-four one year old female Wistar rats (Velaz, Prague, Czech Republic) were used. The animals were housed individually and maintained *ad libitum* access to food and drink according to the design of the experiment. Animals were housed in conventional cages under standard conditions (21-24 °C and 55-65 % humidity) with a 12/12 h light/dark cycle.

Fly locomotor activity measurement. One day prior to the measurement, 30 flies were transferred using CO_2 anesthesia into separate vials with either standard (CTRL), caffeine (CAFF) or cola (COLA) medium. Flies were allowed to feed for 24 hours. The next day, each fly was put into a clean Petri dish with a 5.5 cm diameter and 1.5 cm height without anesthesia. The Petri dish was immediately closed and a video of the fly movement was recorded for 3 minutes using a C922 Pro Stream Webcam (Logitech, Lausanne, Switzerland) camera positioned on a tripod over the Petri dish. Since locomotor activity may vary based on circadian rhythm, in order to minimize the bias in this aspect, all flies were tested in a 4-hour time period starting in the morning. The total moved distance was analyzed using EthoVision XT 10.0 (Noldus Information Technology, Wageningen, Netherlands).

Caffeine and cola intake of rats. After a 7-day period of acclimatization, animals were randomly assigned to experimental groups: group drinking Coca-Cola beverage (COLA), group drinking caffeine solution (CAFF), group drinking tap water (CTRL). Coca-Cola was bought at a local store and it was decarbonated by placing a bottle on a magnetic stirrer for 15 minutes to avoid spilling. Caffeine solution was prepared from caffeine powder (Sigma Aldrich, Missouri, USA). The concentration of the caffeine solution was determined by a preliminary experiment and set in a way animals would get an equal dose of caffeine in both COLA and CAFF groups (approximately 80 mg/kg/24 hours). The actual caffeine dose was subsequently calculated based on liquid intake. Twenty-four hours prior to behavioral testing, animals had replaced their drinking water with *ad libitum* access to the liquid according to their experimental group (cola, caffeine solution, water).

Behavioral testing of rats. To evaluate locomotor activity rats were placed individually in the central zone of cages to monitor their behavior for 3 hours. Total distance moved and velocity were measured to assess locomotor activity. Tests were carried out in a dimly lit room. The behavior was recorded using a specialized PhenoTyper® (Noldus, Wageningen, Netherlands) cage with a dedicated camcorder and recordings were analyzed using the video processing system EthoVision XT 10.0 (Noldus Wageningen, Netherlands). The behavioral testing was performed at the same time during the day to avoid the effect of circadian rhythm.

Statistical analysis was conducted using GraphPad Prism version 6.01 (GraphPad Software, La Jolla, CA, USA). Normality of data was checked using Shapiro–Wilk normality test. Data were analyzed using Student's t-test in case of two datasets or by one-way ANOVA with Bonferroni *post hoc* test when comparing three groups. Data are shown as mean + standard deviation.

There were significant differences in distance moved by flies ($F=6.10$, $p<0.01$, Fig. 1A). Distance moved by COLA group was 2-fold higher than distance moved by CAFF group ($t=3.47$, $p<0.01$, Fig. 1A). There were no differences between CAFF ($t=1.31$, $p=0.61$, Fig. 1A) or COLA ($t=2.10$, $p=0.14$, Fig. 1A) compared to the CTRL group.

For rat experiment, caffeine dose per 24 hours was calculated based on liquid intake. Rats drank less of water ($p<0.001$, $t=7.386$) and caffeine solution ($p<0.001$, $t=6.477$) compared to cola ($F=32.11$, $p<0.001$, Fig. 2A). The relative caffeine dose was not different between the groups, on average 80 mg/kg/24h ($p=0.53$, Fig. 2B). Acute cola intake had a slight effect on total distance ran by rats during 3 hours of observation ($F=2.79$, $p=0.08$, Fig. 1B). The total distance was 2-fold higher in COLA group in comparison to CTRL ($t=2.70$, $p<0.05$, Fig. 1B). The difference between CAFF and COLA group was marginally non-significant ($t=2.52$, $p=0.06$, Fig. 1B). There was no difference between CTRL and CAFF group in locomotor activity ($t=0.26$, $p>0.99$, Fig. 1B).

Our results show that acute cola intake resulted in a higher locomotor activity in both flies and rats in a similar manner. Surprisingly, pure caffeine intake did not lead to higher activity of either flies or rats.

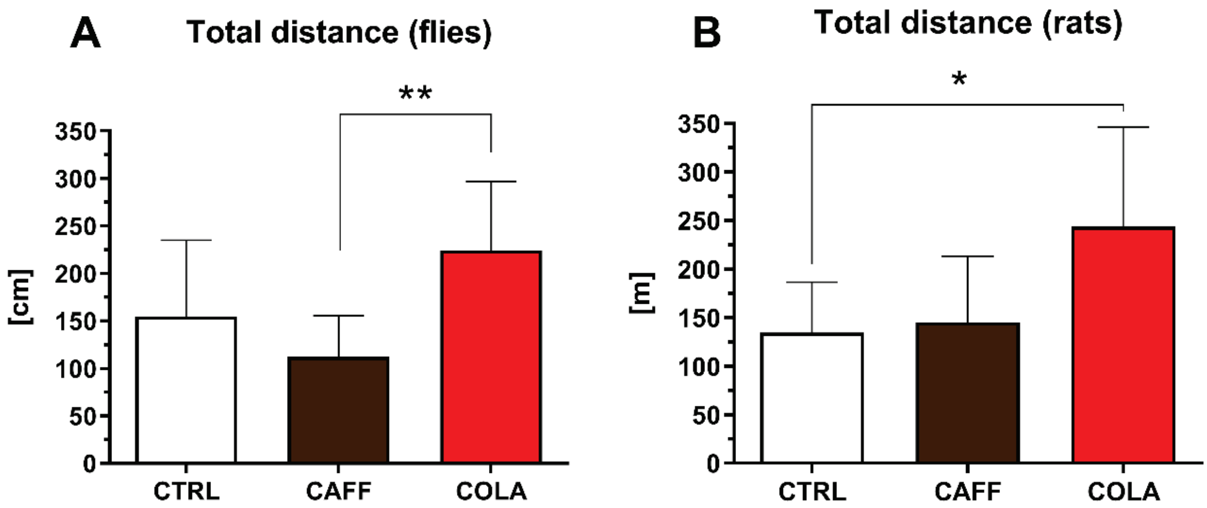

Fig. 1. Locomotor activity after 24 hours of caffeine or cola intake. **A)** Total distance flies moved. **B)** Total distance rats moved. Data shown as mean + standard deviation. CTRL-control group of animals with no intervention; CAFF-animals treated with caffeine solution; COLA-animals treated with cola drink; * $p<0.05$; ** $p<0.01$

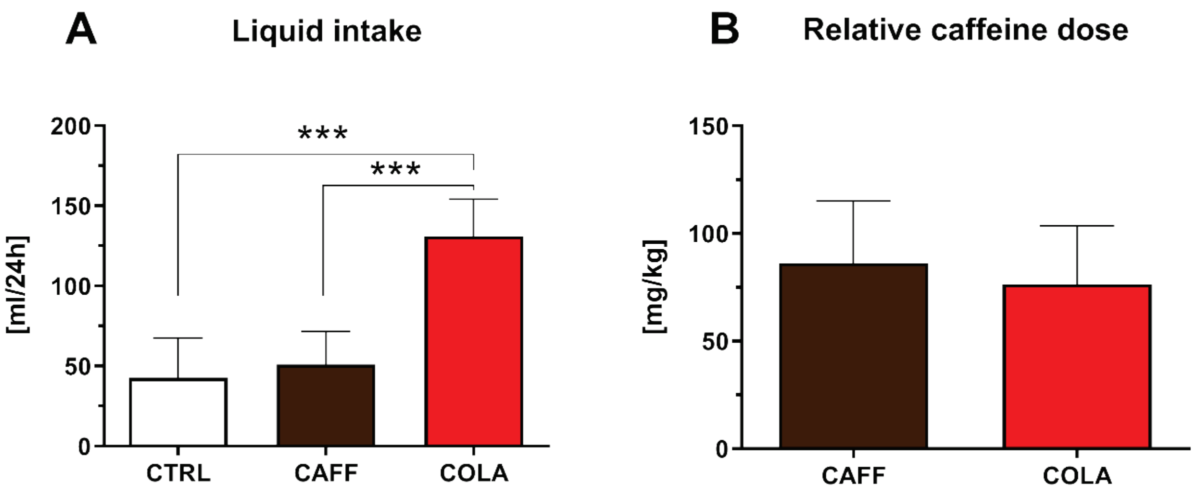

Fig. 2. Caffeine dose received by rats during 24 hours relative to their weight. **A)** Liquid intake of rats during 24h before behavioral testing. **B)** Comparing the dose of caffeine administered to rats, relative to their weight, during 24h before behavioral testing. Data shown as mean + standard deviation. CTRL-control group of rats with no intervention; CAFF-rats treated with caffeine solution; COLA-rats treated with cola drink; *** $p<0.001$

There is evidence indicating that caffeine increases locomotor activity (Ettarh *et al.* 2000, Lin *et al.* 2010). However, we observed increased locomotor activity only after cola intake, not after pure caffeine solution intake. Even though sugar itself does not affect locomotor activity (Flint *et al.* 2006), the synergic effect of sugar and caffeine cannot be ruled out. Clearly, behavioral studies of caffeine, at least in regard to locomotor activity cannot be generalized as an effect of caffeinated beverages. Franklin *et al.* have shown that locomotor activity in rats did not differ after chronic caffeine intake with or without sugar, however the acute effect of such combination may yield different results (Franklin *et al.* 2017). It should be also noted that in our experiments a very low caffeine dose was applied compared to other studies (Baldwin and File 1989, File *et al.* 1988, Hughes *et al.* 2014) which may provide a partial explanation for the contradictory results of various studies. However, the intake of cola in our study was voluntary which makes this dose extremely relevant in regards to the study of behavioral changes.

In this study, we showed that cola but not caffeine, increased locomotor activity in both flies and rats. Whether interaction of caffeine with some other substances contained in cola, particularly sugar, bestows an effect upon behavior in flies or rats, remains to be elucidated. However, we showed that solo study of caffeine cannot represent behavioral effects of caffeinated beverages like cola.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

We would like to thank for support from Comenius University grant [UK/360/2018], KEGA grant 045UK-4/2020 and Dr. Lucia Mentelová (Department of Genetics, Faculty of Natural Sciences, Comenius University) for kindly gifting drosophila stock and for manuscript review.

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