RESEARCH NOTE

Dopamine synergizes with caffeine to increase the heart rate of *Daphnia* [version 1; referees: 1 approved, 2 approved with reservations]

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Abstract

Dopamine is a key neurotransmitter, and is widely used as a central nervous system (CNS) agent. Dopamine plays an important role in humans, including a major role in reward and motivation behaviour. Several addictive drugs are well known to increase neuronal dopamine activity. We selected *Daphnia*, an important model organism, to investigate the effect(s) of selected CNS agents on heart rate. Dopamine's effects on *Daphnia*'s heart has not been previously reported. Caffeine is a well-known and widely consumed stimulant. Ethanol is well known for its effects on both neurological and physiological processes in mammals. We tested the effect of dopamine on the heart rate of *Daphnia*, and compared its effect with caffeine and ethanol alone and in combination. Both caffeine and dopamine were found to instantly increase the heart rate of *Daphnia* in a dose-dependent manner. Interestingly, caffeine synergized with dopamine to increase *Daphnia*'s heart rate. As ethanol decreased the heart rate of *Daphnia* and dopamine increased the heart rate of *Daphnia,* we wanted to test the effect of these molecules in combination. Indeed, Dopamine was able to restore the ethanol-induced decrease in the heart rate of *Daphnia*. Effects of these CNS agents on *Daphnia* can possibly be correlated with similar effects in the case of mammals.

Keywords

Dopamine, heart, neurotransmitter, cardiac, central nervous system

Open Peer Review

Referee Status: ✔ ? ?

	Invited Referees 1	2	3
version 1 published 01 Mar 2018	✔ report	? report	? report

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Author roles: Kundu A: Methodology, Validation, Writing – Original Draft Preparation; **Singh G**: Supervision, Validation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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First published: 01 Mar 2018, **7**:254 (doi: 10.12688/f1000research.12180.1)

Introduction

Neurotransmitters are the key mediators of communication between nerve cells. Because of their effect on brain and spinal cord, central nervous system (CNS) agents can be used to control or treat variety of medical conditions[1]. Stimulation of the hypothalamus can lead to cardiovascular disturbances, indicating a direct connection between the heart and the CNS[2,3]. Different types of rewards are known to increase the level of dopamine in the brain[4]. *Daphnia* are small crustaceans commonly known as "water fleas", and are found in water bodies[5]. *Daphnia* is an ideal organism for research, as it has short life span, and can easily be cultured[6]. These organisms can feed on algae, yeast and bacteria[5]. More importantly, *Daphnia* are transparent, thus allowing clear visualization of different organs, including the heart[7]. The organs are protected by a thin membrane that allows the penetration of different compounds; therefore assisting with heart rate monitoring in real time[5]. Using a microscope that has computer-aided real-time imaging capabilities, the effect of various compounds can be observed on *Daphnia's* heart in real time. *Daphnia's* life span is 40–50 days, which varies in different species and also changed with environmental conditions, especially temperature. Male and female *Daphnia* can easily be differentiated, as female *Daphnia* have brood pouch that holds eggs. These eggs develop into embryos, leading to the production of juveniles that attain sexual maturity within ten days.

Dopamine is important for normal cardiopulmonary response to exercise and is necessary for optimal high-intensity exercise performance. Blocking dopamine receptors appears to be detrimental to exercise performance[8]. Caffeine, by antagonizing adenosine A2A receptors, is known to augment dopamine signalling in the brain[9,10]. Even at routine doses, caffeine can enhance dopamine receptor accessibility in the mammalian CNS[10]. Caffeine has also been reported to normalize the heart rate of *Daphnia*, which is decreased by atropine and atenolol[11]. Ethanol is known to cause progressive weakness, difficulty in walking, and lowered heart rate[12]. Ethanol also inhibits calcium dependent neurotransmitter release, and, excitatory and inhibitory postsynaptic potentials in cultured spinal cord neurons[13].

The aim of the present study was to investigate the effect of Dopamine on *Daphnia's* heart rate, alone and in combination of caffeine and ethanol. The rationale behind this research was that both caffeine and ethanol are known to affect nervous system functions[14], and dopamine is a major neurotransmitter.

Methods

Daphnia culture

Daphnia were isolated from Chitti Vai river of Punjab. For the isolation of *Daphnia*, 0.5–2.0 litres of river water was collected and transported to laboratory. Adult *Daphnia* were manually identified as per the standard identification features[15], and filtered out using muslin cloth. These adults were cultured in 300 ml glass jars containing river water that was filtered with muslin cloth. *Daphnia* culture was supplemented with 0.5% yeast culture, added every third day. Yeast culture, in this case, was used as a food for *Daphnia*. Algae, yeast or bacteria are preferred food for *Daphnia*. Although, many workers use river water for *Daphnia* culture presuming that it would have better mineral composition, in our case, we were also able to culture *Daphnia* in aged tap water in the similar manner. Cultures were routinely monitored to ensure production of healthy *Daphnia*.

Counting of *Daphnia's* heart rate

To investigate the effect of certain agents on the heart rate of *Daphnia*, real-time monitoring of changes in the heart rate of *Daphnia* is required. We used a microscope equipped with computer-aided real-time imaging capability (Magnus Live usb camera viewer, version 2.0, Magnus Analytics, New Delhi-110044, India), and for each reading heart rate was initially counted without any treatment (control). Subsequently, changes in the heart rate was monitored after the addition of selected agents. Each *Daphnia* was placed on the glass slide with 100 ul of water. The slide was observed in real time under the microscope at 40x or 100x magnification, and heart rate was counted. To avoid the effect of temperature or other environmental factors, counting was done after five seconds of starting the microscope. Subsequently, the microscope was switched off for five seconds, cardiovascular agents were added (see Table 1), and heart rate was counted again.

Statistical analysis

A paired t test analysis was done to compare changes in heart rates upon treatment with different agents. Statistical analyses were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA). $P<0.05$ was considered significant.

Results and discussion

Dopamine, like caffeine, increases the heart rate of *Daphnia* in a dose-dependent manner

Dopamine's effects on *Daphnia*'s heart has not been reported previously. We hereby report that dopamine instantly increases the heart rate of *Daphnia* in a dose-dependent manner, and a significant increase (25.7%) in the heart rate was observed, even at a low dose of 0.8 mg/ml (Figure 1). Caffeine showed a similar

Table 1. Central nervous system agents used.

Name (source)	Concentrations
Caffeine (Loba Chemie Pvt Ltd, Mumbai, India)	0.08 to 0.32 mg/ml
Dopamine (Amrit Pharmaceuticals, Aurangabad, India)	0.4 to 3.2 mg/ml
Ethanol (Himedia Laboratories, Mumbai, India)	2–8%

effect on *Daphnia*'s heart rate at a 10-times lower concentration than dopamine (28.5% increase at 0.08 mg/ml, Figure 2). Dopamine is the precursor of norepinephrine, and has been shown to augment heart activity by affecting beta-adrenergic receptors, in the case of a canine model[16]. Furthermore, dopamine can cause both relaxation and contraction of vascular smooth muscle. Dopamine is also known to augment heart activity, pulmonary pressure, and cardiac index in the case of normal and hypertensive individuals[17].

Dopamine synergizes with caffeine to increase the heart rate of *Daphnia*

Caffeine, in combination with dopamine, increased *Daphnia*'s heart rate more than when the agents were administered

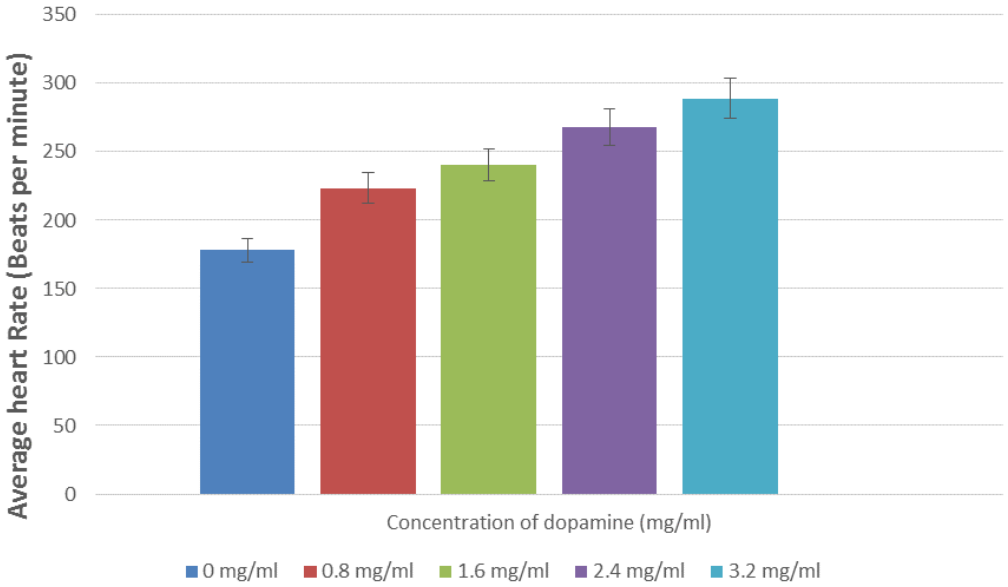

Figure 1. Dopamine increases the heart rate of *Daphnia* in a dose-dependent manner. This experiment was performed two times, and a paired t test analysis vs control indicated the following P values: 0.0070 (for 0.8 mg/ml), 0.0255 (1.6 mg/ml), 0.0424 (2.4 mg/ml), and 0.0344 (3.2 mg/ml). These values are statistically significant.

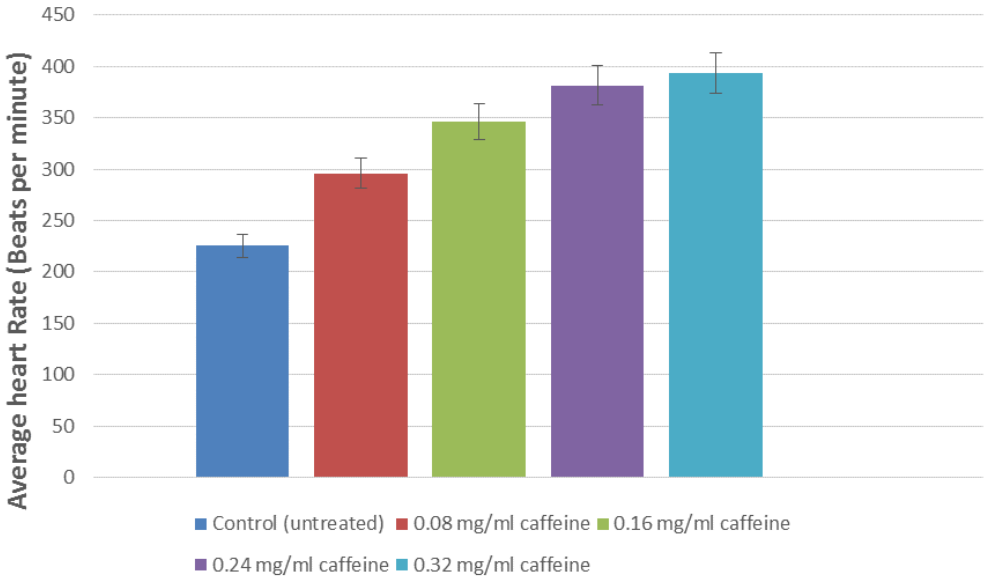

Figure 2. Caffeine increases the heart rate of *Daphnia* in a dose-dependent manner. This experiment was done two times, and a paired t test analysis vs control revealed the following P values: 0.0406 (0.08 mg/ml), 0.0263 (0.16 mg/ml), 0.0367 (0.24 mg/ml), and 0.0189 (0.32 mg/ml). These values are statistically significant.

alone, which suggests a synergistic activity (Figure 3). Dopamine has also been previously reported to play a role in the responses of *Drosophila* to cocaine, nicotine or ethanol[18].

Dopamine overcomes an ethanol-induced decrease of the heart rate of *Daphnia*

To see the effect on the heart rate of *Daphnia*, ethanol was used at a concentration ranging from 2–8%, and was found to decrease the heart rate of *Daphnia* in a dose-dependent manner (Figure 4).

We observed that dopamine was able to rescue the ethanol-induced decrease in the heart rate of *Daphnia*, even at a concentration of 0.4 mg/ml (Figure 5).

Dataset 1. Effect of dopamine, caffeine and ethanol on the heart rate of *Daphnia*

http://dx.doi.org/10.5256/f1000research.12180.d194189

Heart rates (beats per minute) was initially counted without any treatment (controls). Subsequently, changes in the heart rate was monitored after the addition of selected agents.

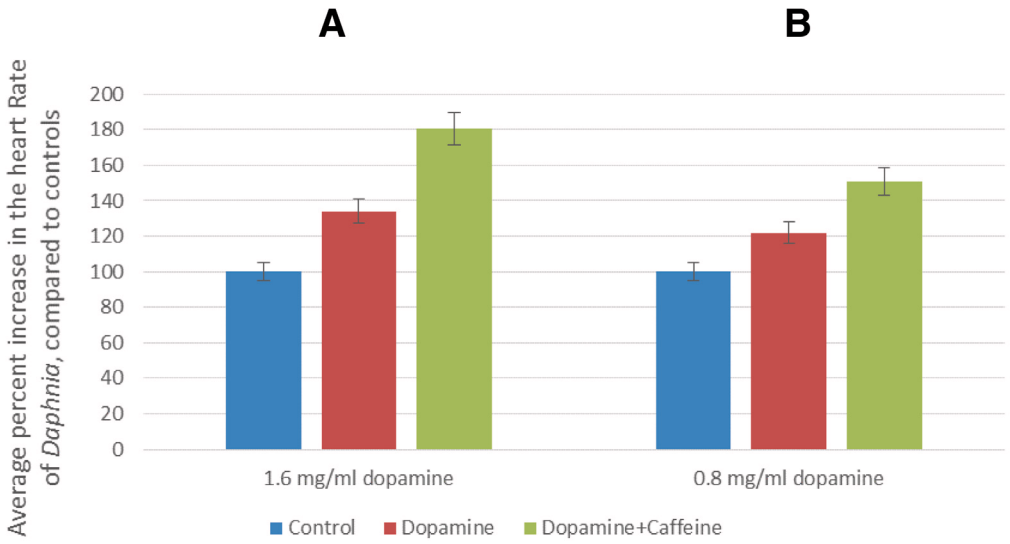

Figure 3. Dopamine synergizes with caffeine to increase the heart rate of *Daphnia*. *Daphnia's* heart rate was measured upon treatment with dopamine alone (red) or a combination of dopamine and caffeine (green). The concentration of caffeine (in combination with dopamine) was (**A**) 40 ug/ml and (**B**) 120 ug/ml. This experiment was performed two times, and a paired t test analysis vs control indicated the following P values: 0.0374 (0.8 mg/ml dopamine) and 0.0230 (1.6 mg/ml dopamine). These values are statistically significant.

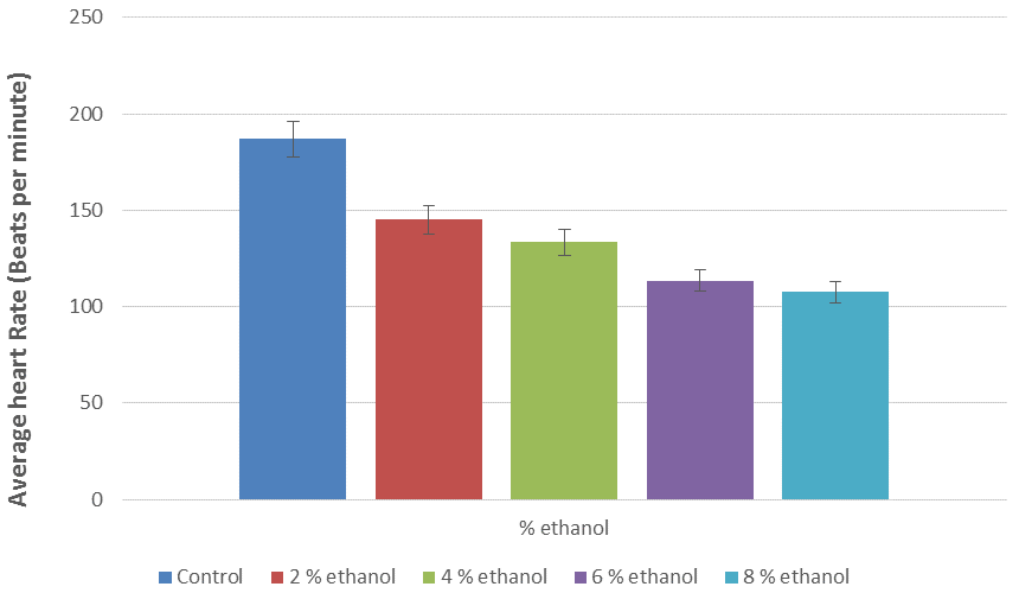

Figure 4. Effect of ethanol on the heart rate of *Daphnia*. This experiment was done two times, and a paired t test analysis vs control indicate the following P values: 0.0152 (2% ethanol), 0.0059 (4% ethanol), 0.0130 (6% ethanol), and 0.0280 (8% ethanol). These values are statistically significant.

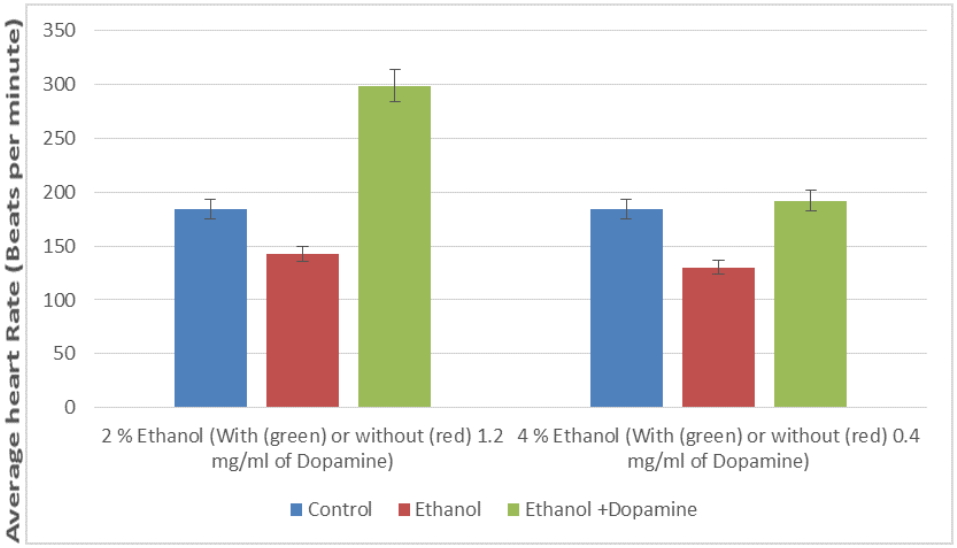

Figure 5. Dopamine overcomes the ethanol-induced decrease on the heart rate of *Daphnia*. At 2% ethanol, dopamine-induced increase in the heart rate was 62.5% compared to control, and 84.8% compared to ethanol-induced heart rate. At 4% ethanol, dopamine-induced increase in the heart rate was 4.3% compared to control, and 33.7% compared to ethanol-induced heart rate.

Conclusion

This fundamental investigation can be of enormous importance, as caffeine and ethanol are the most widely consumed psychoactive drugs, and dopamine is a master neurotransmitter that is known to be involved in variety of diseases[19,20]. It is possible that these psychoactive agents can have similar or more drastic effects in humans. It is, therefore, very important to urgently investigate the effect of these psychoactive agents, alone or in combination, in humans. Such studies can provide crucial information that can be used in a variety of clinical settings. For example, cases of alcohol or caffeine intoxication can be managed by dopamine therapy, treatment(s) of cardiac disorders may be different for alcoholics or coffeeholics, and patients undergoing dopamine therapy need to be regularly monitored for cardiothoracic status, and alcohol/caffeine consumption.

Data availability

Dataset 1: Effect of dopamine, caffeine and ethanol on the heart rate of *Daphnia*. Heart rates (beats per minute) was initially counted without any treatment (controls). Subsequently, changes in the heart rate was monitored after the addition of selected agents. DOI, 10.5256/f1000research.12180.d194189[21]

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Data Source

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Current Referee Status: ✔ ? ?

Version 1

Referee Report 27 June 2018

doi:10.5256/f1000research.13184.r35079

? **Cecilia Scorza**
Department of Experimental Neuropharmacology, Clemente Estable Institute of Biological Research (IIBCE), Montevideo, Uruguay

The study “Dopamine synergizes with caffeine to increase the heart rate of Daphnia” done by Aman Kundu and Gyanesh Singh, investigates the effect of dopamine, caffeine and alcohol on Daphnia’s heart rate. Also the combination of caffeine and ethanol was tested. The rationale of the paper was to use the Daphnia’s heart rate to test treatment(s) of cardiac disorders in high consumers of coffee or alcohol. The importance and objectives of the study are well explained and methods are well described, although some aspects of this manuscript should be really improved and corrected.

The statistical Analysis applied is not correct; consequently I have some doubts if the results would be the same applying a correct statistical analysis. Authors used t-paired test to evaluate a dose-dependence curve in which more than two concentrations are compared. I suggest to authors apply One-Way ANOVA followed by a post hoc test (for example, Newman-Keuls). Additionally, authors indicated that the experiments were done two times. I understand that this means that a duplicate of each experiment were performed, is it correct?. If this is the case, a mean ± SD should be used to compare data. Please, correct.

Another important issue is about the synergism. Author concluded that dopamine synergizes with caffeine to increase the heart rate of Daphnia. But, synergism should be declared if a sub-threshold concentrations of DA and Caff is used. If not, only a potentiation is achieved.
Please, correct the terminology of synergism or other experiments should be done using the combination of sub-threshold doses of DA and Caff.

It would be very important to add positive controls to test Daphnia’s heart rate, for example, noradrenaline or atropine, both are prototypical substances that increase heart rate in vertebrate animals.

In the conclusion: in addition to the purpose of this kind of assay, I wondering if Daphnia’s heart rate assay could be more suitable as a biomarker of toxicity instead of serve as a screening test of different drugs to alter Daphnia’s heart rate?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 13 April 2018

doi:10.5256/f1000research.13184.r31349

Mrinal K. Poddar
Department of Biochemistry, University of Calcutta, Kolkata, India

The original article written by Kundu and Singh focuses on the study of the effects of cardiovascular drugs of the heart rate of Daphnia. In this study the authors have used an interesting heart model of Daphnia.

The article represents the synergistic effect of dopamine and caffeine to increase the heart rate in Daphnia, the tiny water fleas. Though, neither this model can be compare with mammals nor their organ function. The authors have tried to show some effects without mentioning the mechanism of effects. I think they should clarify the followings points:

1. Authors have analyzed the data with a simple t test. Why they didn't they approach with ANOVA test? Moreover, statistical analysis with only two observations is not sufficient. Observations should be made of at least four times for significance test.
2. In Figures, the vertical line given on top of each bar represents what? - SEM or SD?
3. Table 1 shows the lowest concentration of dopamine as 0.4mg/ml which is missing in the entire manuscript. Where is the result of 0.4 mg/ml?
4. Why did the authors choose 40µg or 120µg/ml instead of mg/ml that they have tested and represented in Figure 1. What is the reason of this sudden switch over? Nothing is clear to me!
5. In Figure 3 Why the authors have used the combination of lower concentration of caffeine (40µg/ml) with higher concentration of dopamine (1.6mg/ml) and higher concentration of caffeine (120 µg/ml) with lower concentration of dopamine (0.8mg/ml)? This needs clarification.
6. Figure 5 have the same queries like Figure 3 and it should be better in both the cases of combinations, keep one constant and vary the other one.
7. The Daphnia model does not directly resemble the mammalian system. The conclusion they have

made in the manuscript requires rethinking and should be presented accordingly.
8. In the manuscript the authors didn't mention about the treatment procedure. How have they administered the dopamine, caffeine or alcohol? What is the route of administration of these compounds? Are those administered or given in the culture medium? Depending on the route of administration for this particular model, the author should discuss about the mechanism of action of these agents on Daphnia heart rate or cardiovascular system.

Suggestions:

I think for indexing following works are needed to improve the manuscript:
1. Redesign the experiments as I suggested.
2. More observations (at least 4) for each experiment are needed.
3. Proper statistical analysis is essential. Simple t test is not enough as the experiment are in combination of different agents.

References

1. Kundu A, Singh G: Dopamine synergizes with caffeine to increase the heart rate of Daphnia. *F1000Research*. 2018; **7**. Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 09 March 2018

doi:10.5256/f1000research.13184.r31347

Rafael Antonio Vargas
Departamento de Ciencias Fisiológicas, Facultad de Medicina, Pontificia Universidad Javeriana, Bogotá, Colombia

The original article by Kundu and Singh focuses on the study the effects of cardiovascular drugs on the heart rate of Daphnia. The authors have used an interesting heart model: Daphnia. This model has some advantages in comparison with classical animal models such as rats, mice, dogs, cats, and others. This model could be interesting for researchers from undeveloped countries.
The study shows the effect of dopamine, caffeine, and alcohol on Daphnia heart rate (HR), which has been studied in other animal models. In this case, it is shown that dopamine and caffeine increase HR, and alcohol reduces HR. Interestingly, dopamine restores the low HR ethanol-induced. The authors claim that it's probably similar effects of this agents in humans, however, the Daphnia heart does not represent a direct analog of mammalian cardiac function, so the interpretation of the results of the present study in terms of mammals is problematic.
I have two comments/questions:

1. It is mentioned in the introduction, that atropine and atenolol decrease HR and this is not true in humans: Atenolol, a beta blocker reduces HR, but atropine, a cholinolytic agent, increase HR, so this statement must be revised.
2. It could be useful if the authors add the how HR was performed.

In general, after reading this submission, I consider that this is a short and interesting article about a simple model which will be useful for students, young researchers interested in alternatives to study cardiovascular function. After clarifying the comments the paper can be indexed.

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Cardiovascular physiology, neurophysiology, animal models

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.