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The role of serotonergic signaling on phototactic and locomotor behavior in *Daphnia magna*

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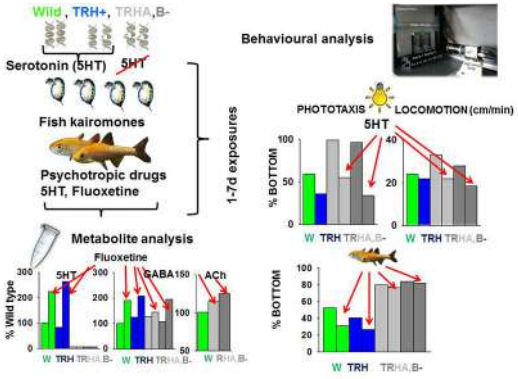
HIGHLIGHTS

- The role of serotonin on *Daphia magna* phototactic behaviour was studied
- TRH- knockout animals moved faster and have an increased negative phototaxis
- Exogenous serotonin recovered the wild type phototaxis in individuals lacking it
- Fish kairomones increased positive phototaxis only in wild type individuals
- TRH knockout animals lack serotonin and have lower levels of dopamine and octopamine

GRAPHICAL ABSTRACT



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ABSTRACT

The role of serotonin in *Daphnia magna* phototactic and locomotor behavior was assessed using reverse genetics and pharmacological treatments with serotonin and fluoxetine. The study was conducted with four clones: the wild type clone and three CRISPR *D. magna* ones with mutations in the tryptophan hydrolase gene (TRH) that is involved in serotonin synthesis. These included clones TRHA− and TRHB− with mutations in both alleles that lack serotonin and the mono-allelic mutant TRH+, that has serotonin. Obtained results indicated that animals lacking serotonin showed an increased negative phototactism and locomotor activity upon light stimuli and a reduced response to fish kairomones relative to the wild type and TRH+ individuals. Exposure to exogenous serotonin re-established the phototactism and locomotor activity of TRH− individuals to those of the wild type but did not affect phototactic responses to fish kairomones. Unexpectedly, fluoxetine was able to modify locomotor activity and phototactic behavior against fish kairomones in TRH− individuals lacking serotonin, and also it increased the concentrations of acethylcholine and GABA in exposed animals, which support the argument that fluoxetine may also affect other neurological pathways.

1. Introduction

Serotonin is an important animal neurotransmitter involved in cognition but also in growth and reproduction (Bacqué-cazenave et al., 2020; Szø et al., 2000). In the crustacean cladoceran species, *Daphnia magna*, we previously found that individuals exposed to the selective serotonin reuptake inhibitor (SSRI) fluoxetine had greater levels of serotonin inmunoreactivity in the brain, reproduced earlier releasing more but smaller neonates (Campos et al., 2016). Exposure to the neurotoxin 5,7-dihydroxytryptamine that selectively destroys serotonergic neurons, reduced serotonin-immunofluorescence and offspring production. The previous results, thus support the argument that serotonin modulates reproduction.

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Drug treatments can act in an unspecific manner in other amines, therefore, genetic models offer a more selective way to explore the consequences of reduced serotonin. Tryptophan hydroxylase is a key enzyme in the synthesis of serotonin. In a previous study by using CRISPR-Cas9 technology we obtained different *D. magna* clonal lineage mutants for the tryptophan hydroxylase gene (Rivetti et al., 2018). Clonal lines with mutations in one of the two alleles of the tryptophan hydroxylase gene (TRH+) had normal levels of serotonin, whereas those having mutations in both alleles showed no detectable levels of serotonin and the opposed phenotype of those exposed to fluoxetine: grew less, reproduced at older ages and produced less and smaller offspring.

Results for the involvement of serotonin on *D. magna* behavior are still inconclusive since disparity of responses has been reported across studies. Rivetti et al. (2015) reported that fluoxetine decreased negative vertical phototaxis in the *D magna* clone F, whereas Simão et al. (2019) reported the opposed responses. The use of different behavioral set ups such as too low and too high light intensities and too short monitoring periods are likely to account for the reported discrepancies. There are also incomplete results about the involvement of serotonin in *D. magna* phototactic behavior against fish kairomones, which is a well and highly studied phenomena that prevents zooplankton to be predated by fish during daylight (Bedrossiantz et al., 2021).

In other crustaceans, such as amphipods, is known that exogenous administration of serotonin or fluoxetine increased positive phototactism and locomotor activity (Bossus et al., 2014; Guler and Ford, 2010). Moreover, it also has been reported that increased locomotor activity, aggression and anxiety behavior in crustaceans can be due to the influence of serotonin on other hormones such as on the Crustacean Hyperglyceamic Hormone, CHH or GABA signaling (Fingerman, 1997; Fossat et al., 2014; Mesquita et al., 2011). Furthermore, in mammalian species and also in humans, therapeutically effects of SSRI occur after prolonged exposures to those chemicals (Reimherr et al., 1998). This means that both exposure time and concentration matters. Indeed in amphipods, phototactic responses to fluoxetine was non monotonic having the greatest effects at 0.01 μg L^{-1} after 1 week of exposure and at 0.01 μg L^{-1} after 1,2 and 3 weeks (Guler and Ford, 2010).

The aim of this work is to study how serotonin regulates phototactic responses of *D. magna* without and with the presence of fish kairomones. To do so we used pharmacological treatments with serotonin and fluoxetine administered at low and high concentrations at short and longer exposure periods combined with the inclusion of mutants lacking serotonin. More specifically we conducted the study with four different *D. magna* clones: The wild type clone (Clone F) that has a marked negative phototactic behavior (Simão et al., 2019) and it becomes more positive when co-exposed with fish kairomones (Bellot et al., 2022). Two clones presenting CRISPR mediated mutations in the two alleles of the tryptophan hydrolase gene enzyme that should lack serotonin (hereafter TRHA−, TRHB−) and a mono-allelic mutant (TRH+) that should have normal levels of serotonin (Fuertes and Barata, 2021; Rivetti et al., 2018).

In an attempt to relate molecular changes to behavioral responses in the four studied clones across serotonin and fluoxetine treatments, we studied 22 neurotransmitters and related metabolites following previous procedures using liquid chromatography coupled to mass spectrometry (Fuertes and Barata, 2021; Gómez-Canela et al., 2019; Rivetti et al., 2019). To monitor changes in phototaxis we used an optimized vertical set up that has been recently used to study how fish kairomones and neuroactive drugs modulate phototactic behavior in different clones of *D. magna* (Bedrossiantz et al., 2021; Bellot et al., 2022).

2. Experimental

2.1. Chemicals and materials

All neurotransmitter standards used for the analytical method and its optimization have been purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and Sigma-Aldrich (Saint Louis, MO, USA). Further information is in Supplementary material.

2.2. Experimental animals and culture conditions

Parthenogenetic cultures of four clones of *D magna* were used as described in the introduction: clones F (wild type), TRH+, TRHA− and TRHB−.

Animals were cultured under a 16 h light: 8 h dark photoperiod cycle, and at 20 ± 1 °C. Ten adult *Daphnia* females were maintained in 2 L of ASTM hard synthetic water (ASTM, 1994) feeding with a food ratio of 500,000 cells/mL of *Chlorella vulgaris* (Barata and Baird, 1998). Cultures were changed with freshly prepare media every other day.

2.3. Exposures and sample collection

Two experiments were performed: the first one (experiment 1) tested how serotonin and fluoxetine modulated phototactic behavior of the studied clones after short and long exposures. The second one (experiment 2) tested the effect of environmental concentrations of fluoxetine and serotonin on phototactic responses to fish kairomones.

In experiment 1, individuals of each clone were exposed at two concentrations of serotonin (10, 100 μg L^{-1}) and fluoxetine (0.1, 40 μg L^{-1}) that best triggered behavioral changes in amphipods and *Daphnia* (Guler and Ford, 2010; Rivetti et al., 2018; Rivetti et al., 2015; Simão et al., 2019). Serotonin and fluoxetine testing solutions were prepared using appropriate aliquots from stock solutions of 1 mg L^{-1} prepared freshly in nanopure water. Measured concentrations of the studies compounds in ASTM water indicated that were stable in water (Bellot et al., 2021). Prior to exposures, 50 neonates < 24 h old of each clone were cultured for a week in 3 L tanks filled with 2 L of media as describes above. The media was replaced every other day. After a week young adults were used for experimentation. In experiment 1, animals were exposed for a week to the tested concentrations in groups of 6 in 200 mL of culture media in 300 mL borosilicate glass vessels. Five replicates per treatment were used. Test media was replaced every other day with freshly prepared one. After 1 day and 7 days, animals were used for behavioral assays. At the end of exposures (7 days) animals of each replicate were collected and pooled in an Eppendorf, snap frozen in liquid N_2 and further stored at −80 °C until processed for metabolic extraction and analysis.

In experiment 2 animals were exposed to 0.1 μg L^{-1} and 100 μg L^{-1} of fluoxetine and serotonin, respectively, for one day with and without fish kairomones following previous procedures (Bedrossiantz et al., 2021) and their behavior was monitored. Obtainment of fish kairomone conditioned water (hereafter named fish kairomone, FK) is described in Supplementary material.

2.4. Behavior swimming assay

At 1 and 7 days of exposure, the locomotion and phototaxis behavior of individuals from the four clones pre-exposed to the studied treatments were assessed using a vertical experimental behavior set up and a 5 min:15 min dark:light photoperiod regimen (Bedrossiantz et al., 2021). We determined the time spent in the bottom virtual zone (%) and the total distance moved (cm) per min. More information is provided in Supplementary material.

2.5. Analysis of neurotransmitters and related metabolites

We studied 22 neurotransmitters and related metabolites following previous procedures using liquid chromatography coupled to mass spectrometer (Fuertes and Barata, 2021). Further details are in Supplementary material.

2.6. Data analyses

Behavioral responses were analyzed by a repeated three or four -way ANOVA design. Each of 20 min monitored in the vertical behavioral set up was considered a repeated measure. Compound, fish kairomone treatments and clone were included as fixed factors. In experiment 1, responses obtained at 1 and 7 days of exposure were analyzed separately. The effects of compound treatments and clone on measured concentrations of the selected metabolites in *D. magna* individuals were analyzed by a two way ANOVA. Prior to analyses, percentage, total distance moved or metabolite concentration data was tested to meet ANOVA assumptions of normality and variance homoscedasticity, and if required, data was arccosine (for %) or log transformed (Zar, 1996). Following ANOVAs, differences among treatments were further compared using post-hoc Tukey's or Dunnett's multiple comparison tests. Analyses were performed with the IBM SPSS Statistics software v27.

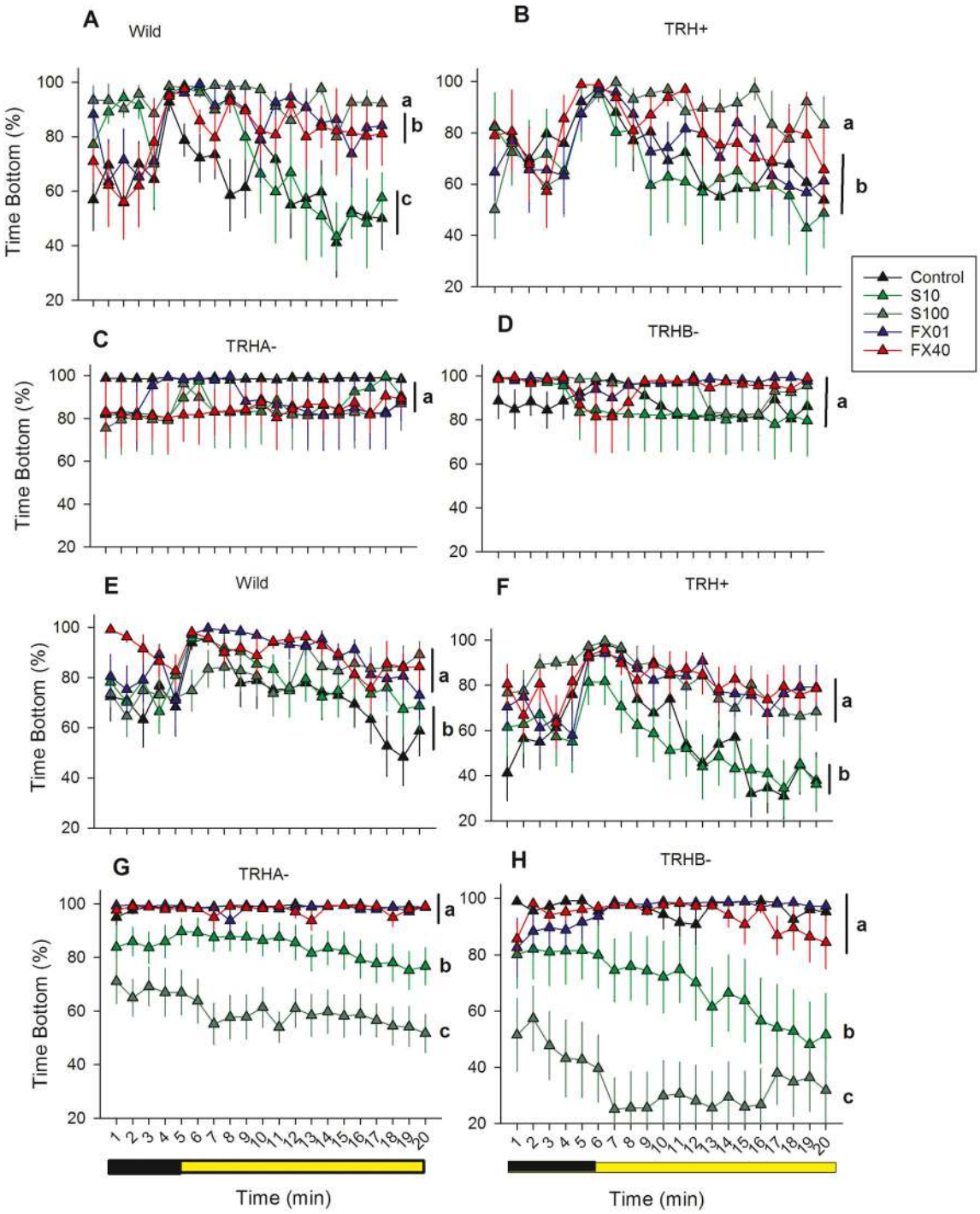


Fig. 1. Phototactic trajectories of the four clones (MEAN ± SE, N = 12) across serotonin and fluoxetine treatments at one (A–D) and seven (E–H) days of exposure. Within each graph, different letters indicated significant ($P < 0.05$) differences among treatments following ANOVA and Tukey's pot-hoc test. S10, S100, FX01, FX40 are 10, 100 μg L^{-1} of serotonin and 0.1, 40 μg L^{-1} of fluoxetine, respectively.

3. Results

3.1. Experiment 1

3.1.1. Phototaxis

Individuals of knockout clones TRHA− and TRHB− swam closer to the bottom (Fig. 1C, D, G, H) than the wild type and TRH+ clones (Fig. 1A, B, E, F). Effects of serotonin and fluoxetine treatments on phototaxis were greater at 7 days (Fig. 1E–H) than at 1 day of exposure and upon light (Fig. 1A–D). In both exposure periods (1 and 7 days) serotonin at high concentrations (100 μg L^{-1}), and the two treatments of fluoxetine, increased negative phototaxis of *D. magna* individuals from the wild type and clone TRH+ (Fig. 1A, B, E, F). At 7 days of exposure individuals from clones (TRHA− and TRHB−) exposed to serotonin increased positive phototaxis in a concentration related manner, whereas fluoxetine treatments did not have any effect. The above described phototactic differences among clones, between dark and light periods and across serotonin and fluoxetine treatments accounted for the reported significant ($P < 0.05$) effects of the repeated measure (phototaxis across the 20 min monitored), clone, treatments and their interactions (Table S4).

3.1.2. Locomotion

Individuals of knockout clones TRHA− and TRHB− swam faster (Fig. 2C, D, G, H) than those from the wild type and clone TRH+ (Fig. 2A, B, E, F), and responded more to the light, serotonin and fluoxetine treatments. The sharp increment and decrement of locomotion activity between min 5 and 6 in Fig. 2 are related to the switching on the light. After one day of exposure to serotonin at 100 μg L^{-1}, individuals of clones

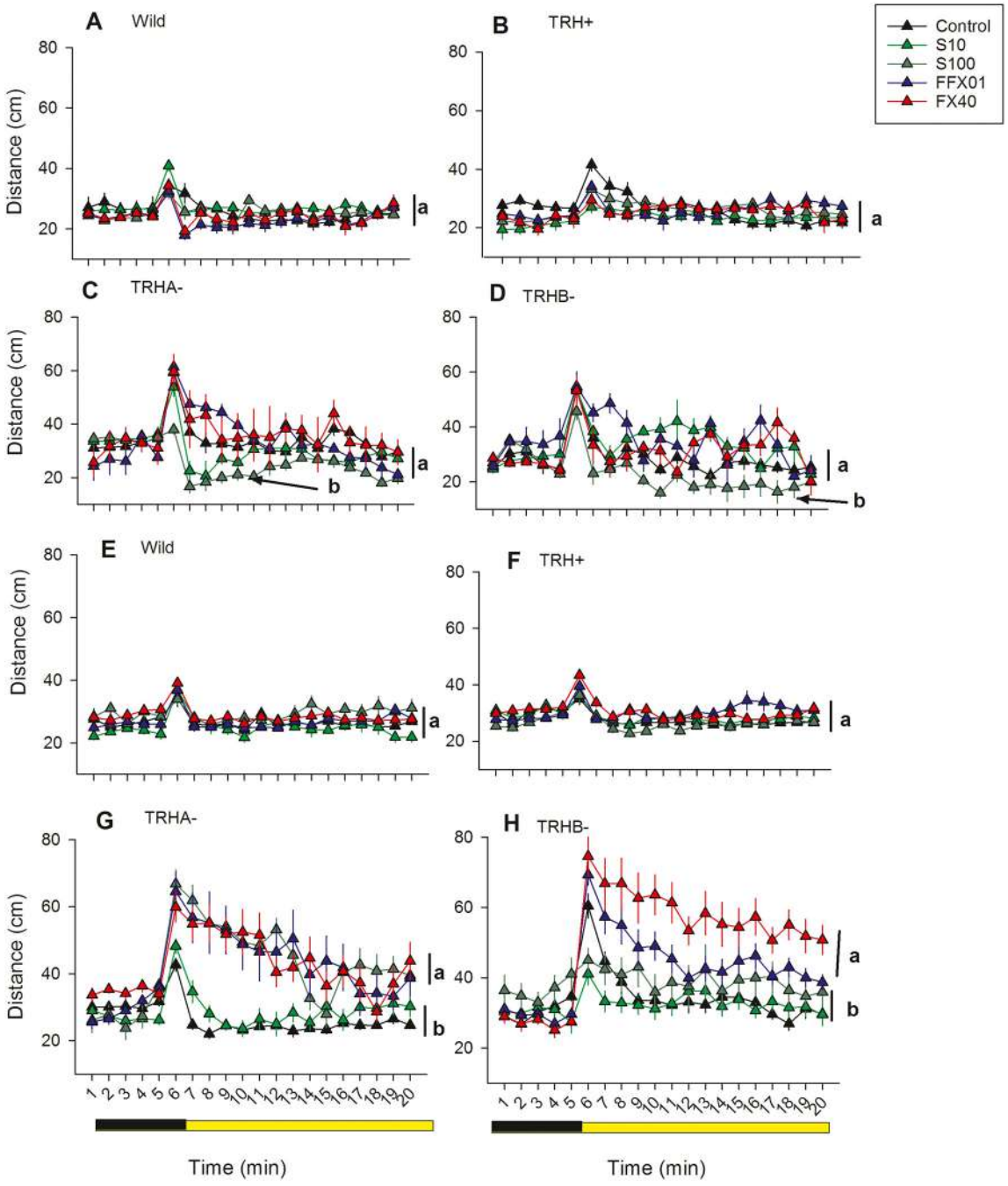


Fig. 2. Locomotor trajectories of the four clones (MEAN ± SE, N = 12) across serotonin and fluoxetine treatments at one (A–D) and seven (E–H) days of exposure. Within each graph, different letters indicated significant ($P < 0.05$) differences among treatments following ANOVA and Tukey's pot-hoc test. Abbreviations are explained in Fig. 1.

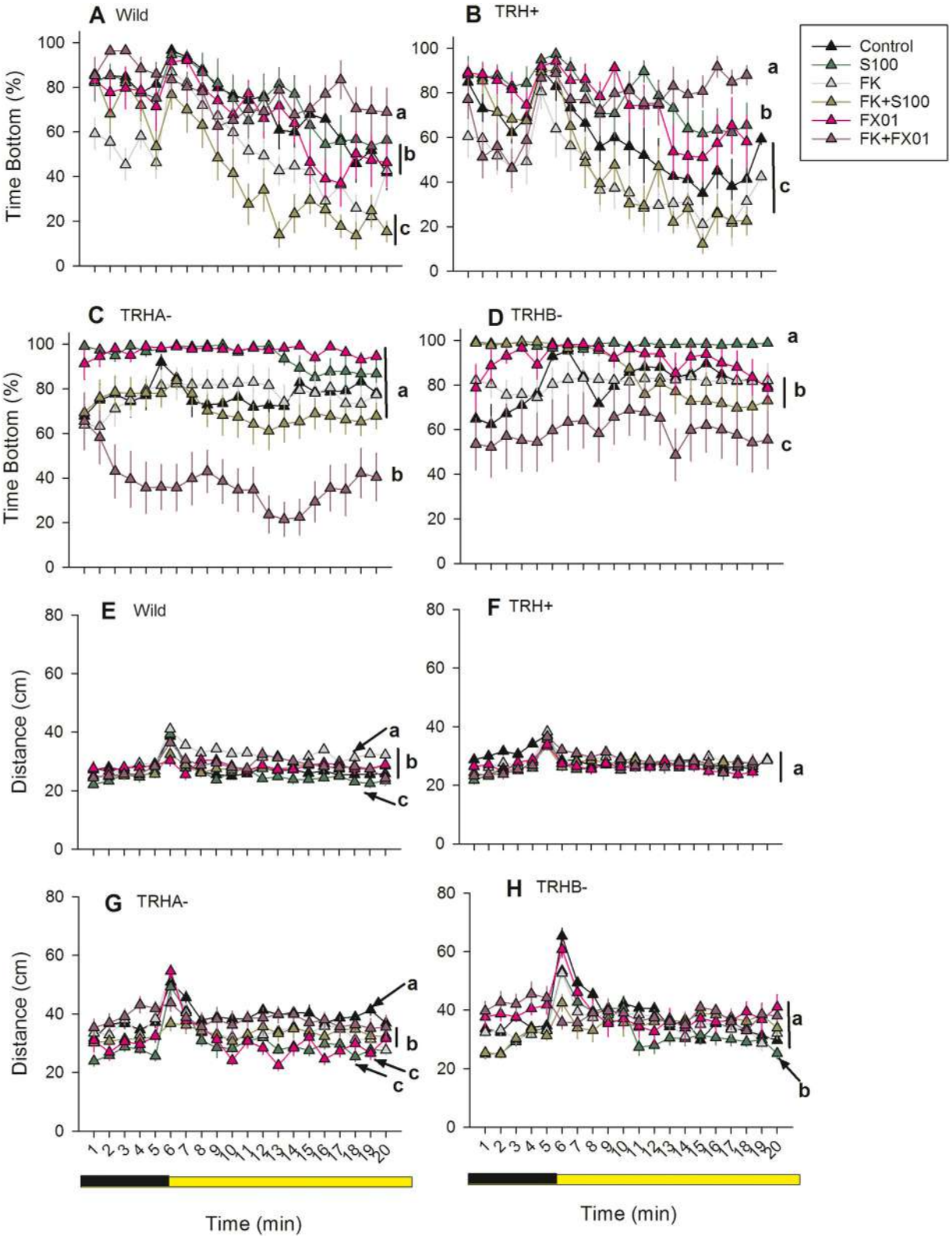
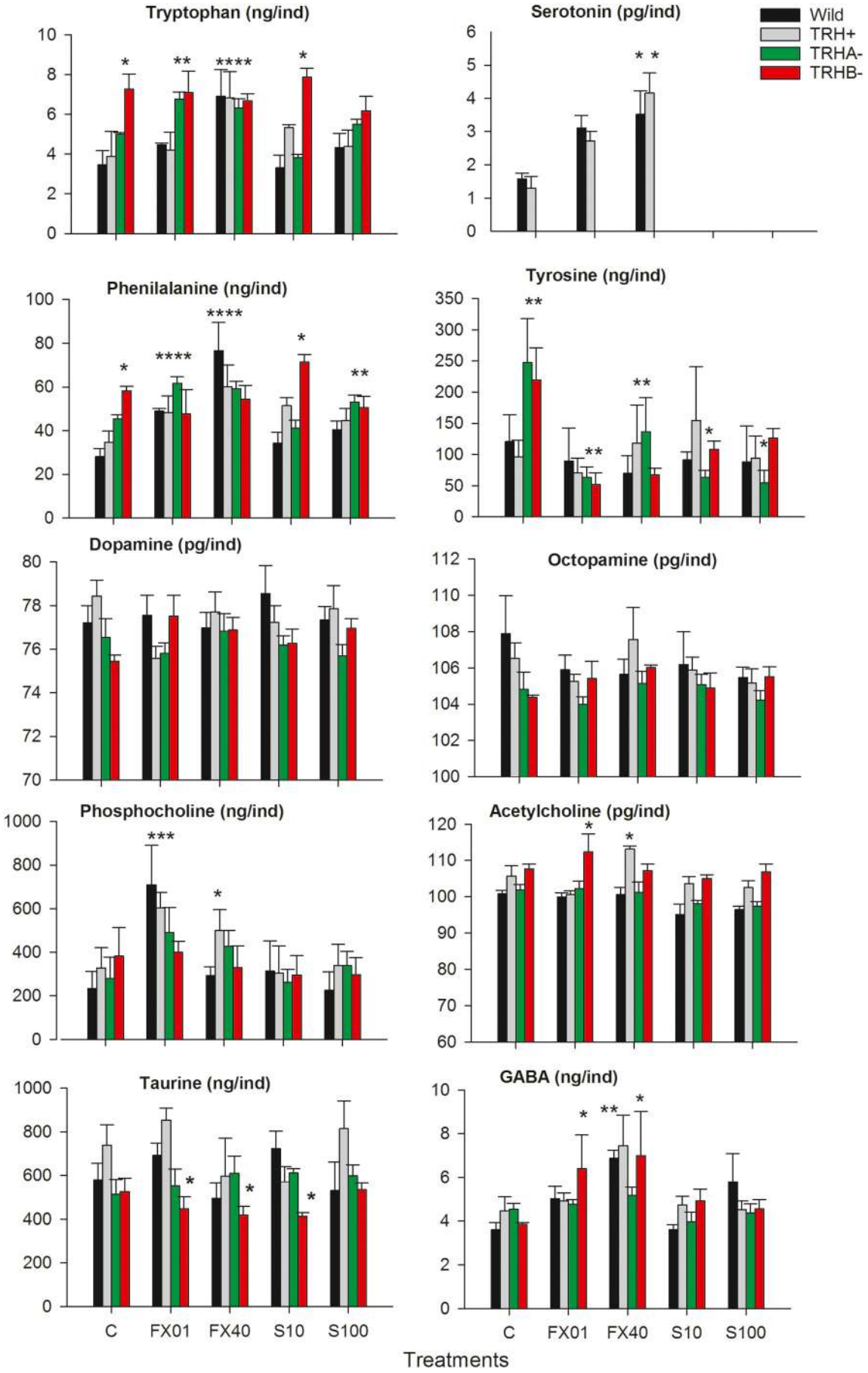


Fig. 3. Phototactic (A–D) and locomotor (E–H) trajectories of the four clones (MEAN ± SE, N = 12) across serotonin, fluoxetine and fish kairomone treatments. Within each graph, different letters indicated significant ($P < 0.05$) differences among treatments following ANOVA and Tukey's pot-hoc test. S100, FX01, FK, FK-S100, FK-FX01 are 100 μg L^{-1} of serotonin, 0.1 μg L^{-1} of fluoxetine, fish kairomone and the fish kairomone co-exposures with serotonin and fluoxetine, respectively.

TRHA − and TRHB − decreased their swimming activity (Fig. 2C, D). At 7 days, both fluoxetine treatments and serotonin at 100 μg L^{-1} increased dramatically the locomotion activity of clones TRHA − and TRHB − upon light. The observed affects between dark and light periods, clones and compounds accounted for the significant main effects and their interaction terms (Table S4).


Tryptophan (ng/ind)
Serotonin (pg/ind)
Wild
TRH+
TRHA-
TRHB-
Phenilalanine (ng/ind)
Tyrosine (ng/ind)
Dopamine (pg/ind)
Octopamine (pg/ind)
Phosphocholine (ng/ind)
Acetylcholine (pg/ind)
Taurine (ng/ind)
GABA (ng/ind)
C
FX01
FX40
S10
S100
Treatments

3.2. Experiment 2

3.2.1. Phototaxis

In a similar way as in experiment 1, serotonin and fluoxetine increased the negative phototactism of individuals from the wild type and TRH+ clone and have little effect on the phototaxis of daphnids from clones TRHA− and TRHB− (Fig. 3A–D). Fish kairomones increased positive phototactism of individuals from the wild type and clone TRH+ but have negligible effects on clones TRHA−, TRHB−. Serotonin had also negligible effects on phototactic behavior induced by fish kairomones in the studied clones. Conversely fluoxetine co-administered with fish kairomones had dramatic effects on phototactic behavior as follows. In individuals of the wild type and clone TRH+, co-exposure to fluoxetine and fish kairomones increased negative phototactism, whereas those from clones TRHA− and TRHB− decreased their negative phototaxis. Clonal differences in the phototactic responses to fish kairomones to serotonin and fluoxetine treatments, and to co-exposures of the previous mentioned compounds with fish kairomones, accounted for the observed significant effects of most factors and their interactions (Table S4).

3.2.2. Locomotion

In a similar way as in experiment 1, individuals of knockout clones TRHA− and TRHB− swam faster (Fig. 3G, H) than those from the wild and clone TRH+ (Fig. 3E, F), and responded more to light. Serotonin at 100 μg L^{-1} alone and co-administered with fish kairomones reduced swimming activity of the studied clones. Fish kairomones increased the activity of the wild type one. The observe affects between dark and light periods, clones, fish kairomones and compounds accounted for the observed significant effects of most factors and their interactions (Table S4).

3.3. Metabolites

Ten out of the 20 metabolites quantified (normetanephrine and epinephrine were not detected) in whole *D. magna* body extracts showed significant differences in their concentrations across clones and treatments or their interactions (Table S5). Selected metabolites are in Fig. 4 and the remaining ones in Table S6. For serotonergic metabolites, internal levels of serotonin were not measured in those individuals treated with exogenous serotonin due to the obvious carry over contamination of the exposure media. As expected, serotonin concentrations were below detection limits for clones TRHA− and TRHB− and then these samples were removed from the statistical analyses. Fluoxetine increased serotonin levels in individuals from the wild type and clone TRH+ in a concentration related manner. Concentrations of tryptophan varied significantly ($P < 0.05$) across clones and treatments: fluoxetine at 40 μg L^{-1} increased tryptophan levels and in most treatments individuals from clone TRHB− and to a lower extent those of clone TRHA− have greater levels. Among the studied dopaminergic metabolites, dopamine and octopamine levels were lower in individuals of clone TRHB−. Serotonin and fluoxetine treatments reduced the levels of tyrosine in clones TRHA−, TRHB−, and increased those of phenylalanine. Individuals of clones TRHA− and TRHB− also had greater levels of phenylalanine. Among cholinergic metabolites, results indicated greater levels of acetylcholine for clone TRHB− and at the high fluoxetine treatment for clone TRH+. Fluoxetine treatments also increased the levels of phosphocholine in individuals from the wild type or clone TRH+. Individuals from lone TRHB− had lower levels of taurine. Levels of GABA were enhanced in individuals treated with 40 μg L^{-1} of fluoxetine and those of TRH+ also at 0.1 μg L^{-1}.

4. Discussion

In both experiments individuals having constitutive levels of serotonin (wild type and TRH+) exposed to serotonin and fluoxetine increased their negative phototaxis right from the first day of exposure. On the contrary, in individuals lacking serotonin, exogenous serotonin exposure after 7 days affected positively phototaxis to a similar extent than wild type organisms in the control treatment. Thus, exogenous serotonin was able to re-establish the original phototactic phenotype in TRH− mutants. These results indicated that both serotonin and fluoxetine act similarly modulating phototaxis. As expected fluoxetine alone hardly affected the phototaxis of TRH− knockout individuals as there was no serotonin to be accumulate (Rivetti et al., 2018). Contrasting behavioral effects of increasing serotonin in animals that constitutively have it may be related to a negative feedback mechanism of receptor signaling pathways to an excess of serotonin for individuals of the wild type and TRH+ clones. This behavior has been found in *Drosophila* (Majeed et al., 2016). Furthermore, serotonin regulates the development of serotonergic neurons and of target tissues (Narboux-Neîme et al., 2013). Thus it is also possible that neurological developmental differences between serotonin knockout *D. magna* individuals may also have accounted for their distinct phototactic responses to exogenous serotonin. Unfortunately there is little information on the effects of serotonin on the phototaxis of crustaceans and other arthropods. The results obtained for knockout TRH− *D. magna* individuals exposed to serotonin agree with those described in other crustaceans and insects such as in a gammarid species and *Drosophila*, in which serotonin decreased their negative phototaxis (Guler and Ford, 2010; Kain et al., 2012). Conversely in honeybees workers, the administration of serotonin decreased their innate positive phototaxis, which agrees with our results obtained for wild type and TRH+ *D. magna* individuals (Thamm et al., 2010).

Individuals from TRH− clones lacking serotonin showed higher locomotor activity and responded to light to a greater extent in both experiments, which agrees with previous studies (Rivetti et al., 2018). Exogenous administration of serotonin and fluoxetine in wild type and TRH+ animals were also able to reduce locomotor activity, which is in line to what have been reported elsewhere in *D. magna* (Heyland et al., 2020) and also in other organism (Bacqué-cazenave et al., 2020). However, fluoxetine dramatically increased the locomotor activity of individuals from clones TRHA− and TRHB− that lack serotonin after 7 days of exposure. These results suggest that fluoxetine may have additional mechanisms of action in *Daphnia*, despite of its primary known one, by which it increases brain and whole organisms serotonin concentrations (Campos et al., 2016; Rivetti et al., 2019). Using a well-established serotonergic response in *Caenorhabditis elegans*, Dempsey et al. (2005) reported that fluoxetine was able to alter egg-laying behavior acting independently either at the serotonin reuptake transporter (SERT) or at serotonin receptors. More specifically the former authors identify that fluoxetine was acting in *C. elegans* via G-protein couple serotonin receptor subtypes. Interestingly *D. magna* TRH− mutants have the transcription of several genes belonging to the previous mentioned signaling receptor route down regulated (Campos et al., 2019). There is also reported evidence that locomotor activity and its response to food in *C. elegans* is regulated by dopaminergic and serotonergic circuits (Sawin et al., 2000). We found in this present study that TRH− clones have lower levels of dopamine, and that fluoxetine increased those levels. Furthermore, fluoxetine was also able to increase the neurotransmitter concentrations of acethylcholine and GABA in mutated TRH− organisms, which have been related with *D. magna* photomotor responses (Bedrossiantz et al., 2020). Thus our results support the findings reported in other model organisms indicating that serotonin control

Fig. 4. Selected neurotransmitter and related metabolites of the four clones (MEAN SE, N = 5) across serotonin and fluoxetine treatments following seven days of exposure. Within each graph, significant treatment and/or treatment x clonal groups ($P < 0.05$) against the wild type control one following ANOVA and Dunnetts post-hoc test are highlighted with an asterisk. For dopamine and octopamine differences among clones have not be highlighted. Abbreviations are explained in Fig. 1. Further explanation is in the text.

both locomotor and phototaxis behavior, but that fluoxetine can act on them also throughout independent mechanisms.

The involvement of serotonin in *D. magna* phototactic behavior to fish kairomones was tested in experiment 2. Results showed that fish kairomones increased positive phototaxis in individuals from the wild type and the TRH+ clones in a similar way as reported previously (Bellot et al., 2022), but hardly affected those individuals lacking serotonin (i.e. TRH−), which suggest that the presence of serotonin is required to trigger *D. magna* responses to fish kairomones. Nevertheless, pharmacological treatments with fluoxetine but not with serotonin modulated phototactic responses of *D. magna* individuals to fish kairomones. Fluoxetine ameliorated fish kairomone effects in wild type or TRH+ individuals but increased those of TRH− individuals. In a different *D. magna* clone that showed opposed and a greater response to fish kairomones than the current one, we reported that neither serotonin nor fluoxetine altered phototactic responses to fish kairomones (Bedrossiantz et al., 2021). Therefore, the results of the present study only partly agree with previous ones, and support the argument that fluoxetine may modulate phototactic responses to fish thought out different mechanisms than serotonin. Nevertheless it is important to consider that fish kairomones may contain infochemicals and other compounds that may affect *D. magna* phototaxis throughout distinct mechanisms. Quite recently several studies have characterized some of the infochemicals that may trigger *Daphnia* phototaxis (Hahn and von Elert, 2022; Hahn et al., 2019; Von Elert and Pohnert, 2000; Von Elert and Stibor, 2006). Future investigations should address which neurological signaling pathways are affected by individual infochemicals.

Results obtained for the studied neurotransmitters and related metabolites are mostly in line with previous studies. Tryptophan is an essential amino acid and also a precursor of serotonin. Individuals of clone TRHB− and to a lower extent those of clone TRHA−, had higher levels of tryptophan and not detectable levels of serotonin. Fluoxetine treatments also increased the levels of serotonin in wild type and TRH+ individuals. These results agree with the reported effects of fluoxetine increasing brain serotonin inmunoactivity in *D. magna* (Campos et al., 2016) and the lack of serotonin in TRH− knockout individuals (Rivetti et al., 2018).

In general dopamine and octopamine levels were lower in TRH− clones. Chemical treatments reduced the levels of tyrosine and increased those of phenylalanine. Phenylalanine and tyrosine constitute the two initial steps in the biosynthesis of dopamine, thus the observed greater levels of dopamine precursors in TRH− clones and lower levels of dopamine and octopamine, may indicate a limited synthesis of these catecholamines. These results may indicate that serotonin may co-regulate the dopaminergic system, which is in line with recent findings in mice (Fischer and Ullsperger, 2017).

For cholinergic metabolites, results indicated greater levels of acetylcholine for clones TRH+ and TRHB− and of phosphocholine in the wild type and TRH+ clones exposed to fluoxetine. There is reported information indicating that fluoxetine interact with the cholinergic system, preventing the release of acetylcholine or blocking nicotinic cholinergic receptors (Chau et al., 2011). Phosphocholine is a precursor of phosphoglycerolipids that are allocated to eggs in reproductive *D. magna* females (Fuertes et al., 2018). Fluoxetine increased reproduction in *D. magna* (Campos et al., 2012), thus it is possible that due to the higher demand in lipids, precursors such as phosphocholine increased (Jordão et al., 2015).

On the other hand, GABA is an important neurotransmitter that has an inhibitory role in the central nervous system. GABA levels increased upon exposure to fluoxetine. There is reported information indication that fluoxetine can enhance GABA (Bhagwagar et al., 2004). Levels of taurine were lower in TRH− clones. In general there is a consensus that taurine is a powerful anti-excitatory neuronal agent that counteract the excitatory effects of L-glutamate stimulation (Foos and Wu, 2002). Taurine together with other neurotransmitters are known to regulate serotonin levels (Becquet et al., 1993), thus it is plausible that clones lacking serotonin may have altered levels of taurine.

5. Conclusions

The reported role of serotonin regulating cognition but also other important processes like feeding, growth and reproduction in both invertebrates and vertebrates, indicates that it modulates neuronal responses necessary for the fine-tuning of key biological processes for life, rather than to trigger them (Bacqué-cazenave et al., 2020; C Rivetti et al., 2018). The results reported here for behavior support that view as the lack of serotonin in TRH− mutants did not prevent them to respond to light, fish kairomones or psychotropic drugs but changed their responses. TRH− individuals lacking serotonin have a more negative phototaxis and higher locomotor activity than wild type or TRH+ individuals that constitutively have normal serotonin levels. The exogenous administration of serotonin re-established the wild type phototactic and locomotor phenotype in TRH− animals. Fish kairomones increased positive phototaxis in Daphnia individuals from the wild type or TRH+ clones but it hardly affected those of TRH− individuals. There were, however, two results that deserved further investigation. Fluoxetine even at low environmental concentrations was able to increase locomotor activity of TRH− animals upon light and its co-administration with fish kairomones also increased substantially their positive phototactism. Fluoxetine was also able to increase GABA and acetylcholine in TRH− mutants, supporting, thus, the argument that fluoxetine may modulate cognitive *D. magna* responses interacting with several neurological signaling pathways.

CRediT authorship contribution statement

Cristian Gómez-Canela: Investigation; Methodology; supervision; Writing. Ferran Esquius: Investigation. Carlos Barata: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; supervision; Writing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159042.

References

ASTM, 1994. Standard guide for conducting renewal life - cycle toxicity tests with Daphnia magna. In: ASTM (Ed.), Annual Book of ASTM Standards, E 1193-94. American Society of Testing and Materials, Philadelphia, PA, pp. 512–518.

Bacqué-cazenave, J., Bharatiya, R., Barrière, G., Delbecque, J.-P., Bouguiyoud, N., Di Giovanni, G., Cattaert, D., De Deurwaerdère, P., 2020. Serotonin in animal cognition and behavior. Int. J. Mol. Sci. 21. https://doi.org/10.3390/ijms21051649.

Barata, C., Baird, D.J., 1998. Phenotypic plasticity and constancy of life-history traits in laboratory clones of Daphnia magna straus: effects of neonatal length. Funct. Ecol. 12, 442–452. https://doi.org/10.1046/j.1365-2435.1998.00201.x.

Becquet, D., Hery, M., Francois-Bellan, A.M., Giraud, P., Deprez, P., Faudon, M., Fache, M.P., Hery, F., 1993. Glutamate, GABA, glycine and taurine modulate serotonin synthesis and release in rostral and caudal rhombencephalic raphe cells in primary cultures. Neurochem. Int. 23, 269–283. https://doi.org/10.1016/0197-0186(93)90118-O.

Bedrossiantz, J., Fuertes, I., Raldua, D., Barata, C., 2021. Pharmacological modulation of fish-induced depth selection in D. Magna: the role of cholinergic and GABAergic signalling. Sci. Rep. 11. https://doi.org/10.1038/s41598-021-98886-w.

Bedrossiantz, J., Martínez-Jerónimo, F., Bellot, M., Raldua, D., Gómez-Canela, C., Barata, C., 2020. A high-throughput assay for screening environmental pollutants and drugs impairing predator avoidance in Daphnia magna. Sci. Total Environ. 740. https://doi.org/10.1016/j.scitotenv.2020.140045.

Bellot, M., Barata, C., Gómez-Canela, C., 2021. Aqueous stability and degradation of psychiatric and neuroactive compounds and its biological activity in Daphnia magna. Sci. Total Environ. 798. https://doi.org/10.1016/j.scitotenv.2021.149252.

Bellot, M., Gómez-Canela, C., Barata, C., 2022. Phototactic behaviour and neurotransmitter profiles in two Daphnia magna clones: vertical and horizontal responses to fish kairomones and psychotropic drugs. Sci. Total Environ. 830. https://doi.org/10.1016/j.scitotenv.2022.154684.

Bhagwagar, Z., Wylezinska, M., Taylor, M., Jezzard, P., Matthews, P.M., Cowen, P.J., 2004. Increased brain GABA concentrations following acute Administration of a Selective Serotonin Reuptake Inhibitor. Am. J. Psychiatry 161, 368–370. https://doi.org/10.1176/appi.ajp.161.2.368.

Bossus, M.C., Guler, Y.Z., Short, S.J., Morrison, E.R., Ford, A.T., 2014. Behavioural and transcriptional changes in the amphipod Echinogammarus marinus exposed to two antidepressants, fluoxetine and sertraline. Aquat. Toxicol. 151, 46–56. https://doi.org/10.1016/j.aquatox.2013.11.025.

Campos, B., Piña, B., Fernández-Sanjuán, M., Lacorte, S., Barata, C., 2012. Enhanced offspring production in Daphnia magna clones exposed to serotonin reuptake inhibitors and 4-nonylphenol. Stage- and food-dependent effects. Aquat. Toxicol. 109, 100–110.

Campos, B., Rivetti, C., Kress, T., Barata, C., Dircksen, H., 2016. Depressing antidepressant: fluoxetine affects serotonin neurons causing adverse reproductive responses in Daphnia magna. Environ. Sci. Technol. 50. https://doi.org/10.1021/acs.est.6b00826.

Campos, B., Rivetti, C., Tauler, R., Piña, B., Barata, C., 2019. Tryptophan hydroxylase (TRH) loss of function mutations in daphnia deregulated growth, energetic, serotoninergic and arachidonic acid metabolic signalling pathways. Sci. Rep. 9, 9. https://doi.org/10.1038/s41598-019-39987-5.

Chau, D.T., Rada, P.V., Kim, K., Kosloff, R.A., Hoebel, B.G., 2011. Fluoxetine alleviates behavioral depression while decreasing acetylcholine release in the nucleus accumbens Shell. Neuropsychopharmacology 36, 1729–1737. https://doi.org/10.1038/npp.2011.54.

Dempsey, C.M., Mackenzie, S.M., Gargus, A., Blanco, G., Ji, Y.S., 2005. Serotonin (5HT), fluoxetine, imipramine and dopamine target distinct 5HT receptor signaling to modulate Caenorhabditis elegans egg-laying behavior. Genetics 169, 1425–1436. https://doi.org/10.1534/genetics.104.032540.

Fingerman, M., 1997. Crustacean endocrinology: a retrospective, prospective, and introspective analysis. Physiol. Zool. 70, 257–269.

Fischer, A.G., Ullsperger, M., 2017. An update on the role of serotonin and its interplay with dopamine for reward. Front. Hum. Neurosci. 11, 484. https://doi.org/10.3389/fnhum.2017.00484.

Foos, T.M., Wu, J.-Y., 2002. The role of taurine in the central nervous system and the modulation of intracellular calcium homeostasis. Neurochem. Res. 27, 21–26. https://doi.org/10.1023/A:1014890219513.

Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Delbecque, J.P., Cattaert, D., 2014. Anxiety-like behavior in crayfish is controlled by serotonin. Science (80-.) 344, 1293–1297. https://doi.org/10.1126/science.1248811.

Fuertes, I., Barata, C., 2021. Characterization of neurotransmitters and related metabolites in Daphnia magna juveniles deficient in serotonin and exposed to neuroactive chemicals that affect its behavior: a targeted LC-MS/MS method. Chemosphere 263, 127814. https://doi.org/10.1016/j.chemosphere.2020.127814.

Fuertes, I., Jordão, R., Casas, F., Barata, C., 2018. Allocation of glycerolipids and glycerophospholipids from adults to eggs in Daphnia magna: perturbations by compounds that enhance lipid droplet accumulation. Environ. Pollut. 242, 1702–1710. https://doi.org/10.1016/j.envpol.2018.07.102.

Gómez-Canela, C., Rovira García, X., Martínez-Jerónimo, F., Marcé, R.M., Barata, C., 2019. Analysis of neurotransmitters in Daphnia magna affected by neuroactive pharmaceuticals using liquid chromatography-high resolution mass spectrometry. Environ. Pollut. 254. https://doi.org/10.1016/j.envpol.2019.113029.

Guler, Y., Ford, A.T., 2010. Anti-depressants make amphipods see the light. Aquat. Toxicol. 99, 397–404. https://doi.org/10.1016/j.aquatox.2010.05.019.

Hahn, M., von Elert, E., 2022. One kairomone and multiple effects in daphnia Species—5α-cyprinol sulfate induces morphological defenses in the invasive species Daphnia lumholtzi. Front. Ecol. Evol. 10. https://doi.org/10.3389/fevo.2022.804521.

Hahn, M.A., Effertz, C., Bigler, L., Elert, E.V., 2019. 5α-cyprinol sulfate, a bile salt from fish, induces diel vertical migration in daphnia. elife 8. https://doi.org/10.7554/eLife.44791.

Heyland, A., Bastien, T., Halliwushka, K., 2020. Transgenerational reproductive effects of two serotonin reuptake inhibitors after acute exposure in Daphnia magna embryos. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 238, 108875. https://doi.org/10.1016/j.cbpc.2020.108875.

Jordão, R., Casas, J., Fabrias, G., Campos, B., Piña, B., Lemos, M.F.L., Soares, A.M.V.M., Tauler, R., Barata, C., 2015. Obesogens beyond vertebrates: lipid perturbation by tributyltin in the crustacean Daphnia magna. Environ. Health Perspect. 123, 813–819. https://doi.org/10.1289/ehp.1409163.

Kain, J.S., Stokes, C., De Bivort, B.L., 2012. Phototactic personality in fruit flies and its suppression by serotonin and white. Proc. Natl. Acad. Sci. U. S. A. 109, 19834–19839. https://doi.org/10.1073/pnas.1211988109.

Majeed, Z.R., Abdeljaber, E., Soveland, R., Cornwell, K., Bankemper, A., Koch, F., Cooper, R.L., 2016. Modulatory action by the serotonergic system: behavior and neurophysiology in Drosophila melanogaster. Neural Plast. 2016, 1–23. https://doi.org/10.1155/2016/7291438.

Mesquita, S.R., Guilhermino, L., Guimarães, L., 2011. Biochemical and locomotor responses of Carcinus maenas exposed to the serotonin reuptake inhibitor fluoxetine. Chemosphere 85, 967–976. https://doi.org/10.1016/j.chemosphere.2011.06.067.

Narboux-Neîme, N., Angenard, G., Mosienko, V., Klempin, F., Pitychoutis, P.M., Deneris, E., Bader, M., Giros, B., Alenina, N., Gaspar, P., 2013. Postnatal growth defects in mice with constitutive depletion of central serotonin. ACS Chem. Neurosci. 4, 171–181. https://doi.org/10.1021/cn300165x.

Reimherr, F.W., Amsterdam, J.D., Quitkin, F.M., Rosenbaum, J.F., Fava, M., Zajecka, J., Beasley Jr., C.M., Michelson, D., Roback, P., Sundell, K., 1998. Optimal length of continuation therapy in depression: a prospective assessment during long-term fluoxetine treatment. Am. J. Psychiatry 155, 1247–1253. https://doi.org/10.1176/ajp.155.9.1247.

Rivetti, C., Campos, B., Barata, C., 2015. Low environmental levels of neuro-active pharmaceuticals alter phototactic behaviour and reproduction in Daphnia magna. Aquat. Toxicol. 170, 289–296. https://doi.org/10.1016/j.aquatox.2015.07.019.

Rivetti, C., Campos, B., Piña, B., Raldúa, D., Kato, Y., Watanabe, H., Barata, C., 2018. Tryptophan hydroxylase (TRH) loss of function mutations induce growth and behavioral defects in Daphnia magna. Sci. Rep. 8, 11. https://doi.org/10.1038/s41598-018-19778-0.

Rivetti, C., Climent, E., Gómez-Canela, C., Barata, C., 2019. Characterization of neurotransmitter profiles in Daphnia magna juveniles exposed to environmental concentrations of antidepressants and anxiolytic and antihypertensive drugs using liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 411, 5867–5876. https://doi.org/10.1007/s00216-019-01968-y.

Sawin, E.R., Ranganathan, R., Horvitz, H.R., 2000. C. Elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. Neuron 26, 619–631. https://doi.org/10.1016/S0896-6273(00)81199-X.

Simão, F., Martínez-Jerónimo, F., Blasco, V., Moreno, F., Porta, J., Pestana, J., Soares, A.M.V.M., Raldúa, D., Barata, C., 2019. Using a new high-throughput video-tracking platform to assess behavioural changes in Daphnia magna exposed to neuro-active drugs. Sci. Total Environ. 662, 160–167. https://doi.org/10.1016/j.scitotenv.2019.01.187.

Szø, J.Y., Victor, M., Loer, C., Shi, Y., Ruvkun, G., 2000. Food and metabolic signalling defects in a Caenorhabditis elegans serotonin-synthesis mutant. Nature 403, 560–564. https://doi.org/10.1038/35000609.

Thamm, M., Balfanz, S., Scheiner, R., Baumann, A., Blenau, W., 2010. Characterization of the 5-HT1A receptor of the honeybee (Apis mellifera) and involvement of serotonin in phototactic behavior. Cell. Mol. Life Sci. 67, 2467–2479. https://doi.org/10.1007/s00018-010-0350-6.

Von Elert, E., Pohnert, G., 2000. Predator specificity of kairomones in diel vertical migration of daphnia: a chemical approach. Oikos 88, 119–128. https://doi.org/10.1034/j.1600-0706.2000.880114.x.

Von Elert, E., Stibor, H., 2006. Predator-mediated life history shifts in daphnia: enrichment and preliminary chemical characterisation of a kairomone exuded by fish. Arch. fur Hydrobiol. 167, 21–35. https://doi.org/10.1127/0003-9136/2006/0167-0021.

Zar, J.H., 1996. Biostatistical Analysis. Third edit. Bioestatistical AnalysisPrentice-Hall International, Inc, New Jersey.