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The serotonin 1A receptor modulates the social behaviour within groups of a cooperatively-breeding cichlid

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ABSTRACT

The neurotransmitter serotonin (5-HT) reduces aggressive behaviour in a number of vertebrates, and the 5-HT$_{1A}$ receptor is known to be involved in this regulation. However, the role of this receptor in the modulation of sociopositive behaviour remains largely unknown. Here we investigated the role of the 5-HT$_{1A}$ receptor in the regulation of aggressive, submissive and affiliative behaviour in the cooperatively-breeding cichlid *Neolamprologus pulcher*. In two experiments, we performed intramuscular injections of a 5-HT$_{1A}$ agonist (8-OH-DPAT) and antagonist (Way-100635) followed by recordings of social behaviour of injected fish within their social groups. We determined the concentrations and post-injection times when the drugs had the greatest effect on social behaviour. We recorded spontaneous social behaviour in both experiments. In the second experiment we also recorded behaviour after social groups received a territorial challenge by live presentations of either conspecifics or egg predators. The 5-HT$_{1A}$ agonist caused an increase in aggression and a decrease in submission and affiliation, whereas the antagonist had the opposite effects. Thus, the 5-HT$_{1A}$ receptor plays an important regulatory role not only for aggressive but also sociopositive behaviour.

1. Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is one of the major neurotransmitters in the central nervous system, which modulates the expression of social behaviour (reviewed in: Lillesaar, 2011; Lucki, 1998; Puglisi-Allegra and Andolina, 2015). In many vertebrates and invertebrates, including mice (Lopez-Mendoza et al., 1998), lizards (Deckel, 1996), fish (Clotfelter et al., 2007) and lobsters (Kravitz, 2000), higher levels of serotonin reduce levels of aggression (Edwards and Kravitz, 1997). In contrast, the regulation of sociopositive behaviour by serotonin has rarely been studied. However, results from rainbow trouts (*Oncorhynchus mykiss*) and lizards (*Anolis carolinensis*) indicate that serotonin and subordination may be positively associated (Larson and Summers, 2001; Winberg and Lepage, 1998), and that affiliative behaviour is reduced by serotonin in titi monkeys (*Callicebus cupreus*) (Larke et al., 2016).

The effects of serotonin on social behaviour are mediated by its effects on behavioural and physiological functions, including appetite, locomotion, fear and stress. Serotonin plays a key role in the endocrinal regulation via the serotonergic projections from the raphe nucleus to the hypothalamus (Barosky et al., 1983; Prasad et al., 2015; Winberg and Thörnqvist, 2016), for instance regulating prolactin release (Barosky et al., 1983). Serotonin, together with thyroid-hormone and nerve growth factor-inducible factor A (NGFI-A), mediates maternal effects on the offspring's expression of hippocampal glucocorticoid receptors (GR) (Hellstrom et al., 2012).

After being released into the synaptic cleft, serotonin acts through binding to different, mostly postsynaptic, receptors. Three of these receptors, namely 1A, 2A and 7, have been related to mood in fish and mice (fish: Clotfelter et al., 2007; Paula et al., 2015; mice: Romano et al., 2014; Zhang et al., 2004). In this study, we focus on the 5-HT$_{1A}$ receptor, because of its established role in the modulation of several social behaviours. Its activation has attenuated aggression during contests with conspecifics or the own mirror images of several vertebrates (e.g. Chaouloff et al., 1999; Clotfelter et al., 2007; Kiser et al., 2012; Lopez-Mendoza et al., 1998). One study in the cleaner fish *Labroides dimidiatus* reported modulatory effects also on cooperative behaviour (Paula et al., 2015). However, it is yet unknown, whether the effects of the 5-HT receptor on social behaviour depend on the degree the receptor is activated or blocked, or on the social context in which it is applied. Moreover, the role of the receptor for sociopositive behaviour, such as submissive and affiliative behaviour has hardly been addressed (but see

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Larke et al., 2016).

Here we aim to answer the following questions. (1) How does the modulation of social behaviour by the 5-HT_{1A} receptor depend on the dosage of receptor agonist and antagonist, and when do they take effect on behaviour (Experiment 1)? (2) Do the effects of this receptor on aggression depend on social and ecological context, that is, does the receptor affect aggression differently when shown towards group members (Experiments 1 and 2) or different types of territory intruders (Experiment 2)? (3) Is the 5-HT_{1A} receptor involved in the modulation of sociopositive behaviour (Experiments 1 and 2)? To answer these questions, we performed two experiments. (1) We tested the effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT and the antagonist Way-100635 on spontaneous social behaviour within social groups of the cooperatively-breeding cichlid *N. pulcher* (Table S1), varying the dosages of the drugs and the times when the effect on behaviour were observed. (2) We tested the effects of agonist and antagonist on cooperative territory defence experimentally elicited by simulated intrusions of conspecific competitors and of egg predators, respectively.

Cooperative breeding of *N. pulcher* has evolved in response to the extraordinarily high predation pressure, forcing the fish to form groups in order to successfully defend their offspring from predation (Groenewoud et al., 2016). Dominant and subordinate group members cooperate in jointly defending the territory against fish and egg predators as well as conspecific and heterospecific space competitors. The defence behaviour by subordinate helpers is part of the 'rent' the latter pay in exchange for being tolerated by dominants at a territory ('pay to stay', Fischer et al., 2014, Taborsky, 2016). Subordinates can also appease the aggression by dominants and achieve tolerance by enhancing their submissive display behaviours (Bergmüller and Taborsky, 2005; Fischer et al., 2014, 2017; Taborsky et al., 2012). Expressing helping behaviour in form of defence and expression of submission are negatively correlated among subordinate individuals (Fischer et al., 2017; Kasper et al., 2017, 2018a) and represent alternative behavioural types (Kasper et al., 2018b).

1.1. Predictions

Based on the previous findings in Siamese fighting fish, *Betta splendens* (Clotfelter et al., 2007), we predicted that after injection with the agonist 8-OH-DPAT focal *N. pulcher* will show less aggressive behaviour against other group members as well as against the conspecific intruder and egg predator. The latter is expected, because even though defence against egg predators by subordinates is functionally part of the rent they pay towards dominants, mechanistically it involves aggressive motivation. However, we predicted that the effect of the drugs on aggression is stronger in the presence of a conspecific intruder (i.e. a competitor) than in the presence of an egg predator, as conspecific intruders pose a direct risk for a subordinate's position in the social hierarchy of a group. Opposite effects are expected after injection of the antagonist Way-100635 (Clotfelter et al., 2007; Paula et al., 2015). We further predicted that injection of the agonist will increase the expression of submissive displays in *N. pulcher* (see Larson and Summers, 2001), whereas the antagonist should lead to a reduction of submission. Affiliative behaviour comprises a suite of distinct displays in *N. pulcher*, such as a subordinate softly touching the belly of a dominant fish or swimming in parallel with it, which serves to establish socio-positive contact. We predicted that serotonin agonist will reduce affiliative behaviour, as it has been previously shown in titi monkeys (*Callicebus cupreus*; Larke et al., 2016). For the different intruder types, we predicted the effect of the drug on sociopositive behaviour in the presence of intruders to be weaker as compared to the control treatment without intruders. Sociopositive behaviour towards dominants is critically important to retain acceptance in a group, but acceptance by dominants can also be achieved by helping behaviour such as joining in territory defence. Thus in the absence of territorial intruders (control treatment), helpers can appease dominants solely by sociopositive behaviour, in particular submission (Bergmüller and Taborsky, 2005; Fischer et al., 2017) and therefore the drug effects should be stronger during the control treatment.

2. Methods

2.1. Study species

N. pulcher is a cooperatively-breeding cichlid species endemic to Lake Tanganyika, East Africa. This species lives in groups consisting of one breeder pair and between one and up to 20 helpers (Taborsky, 2016). While all subordinate, juvenile group members act as brood care helpers at the natal territory, after reaching sexual maturity offspring may either disperse into other groups where they join again as brood care helpers or they become independent breeders, or they may continue to stay at the natal territory as helper and try to inherit the breeder position there (Jungwirth et al. subm.). Duties of helpers include direct brood care of eggs and larvae (cleaning and fanning for oxygen provision), and territory maintenance and defence (Taborsky, 2016). In return for help they are tolerated by the dominant breeders at the group's territory and thereby gain access to shelter and protection from predation ('pay-to-stay'; Zöttl et al., 2013, Fischer et al., 2014). They have a linear size-based hierarchy with the largest fish being the breeder male, which is dominant over the breeder female followed by the helpers ranked by their size (Taborsky, 2016). To maintain a stable hierarchy, *N. pulcher* use a range of finely graded submissive, affiliative and aggressive displays when interacting with group members (Table S1) (Taborsky, 2016).

2.2. Study subjects and housing conditions

All experimental procedures were approved by the Veterinary Office of the Kanton Bern, Switzerland, licence number BE 93/18, and were carried out in accordance to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, USA as well as the EU Directive 2010/63/EU for animal experiments. All cichlids used in the experiments were bred and housed at the Ethological Station Hasli of the Institute of Ecology and Evolution, University of Bern, which is a licensed breeding facility for cichlid fish (licence number BE 4/11, Veterinary Office of the Kanton Bern). In total, 17 social groups of four fish were established consisting of a breeder pair and two helpers. In Experiment 1, small helpers measured 3.29 ± 0.04 cm (mean ± se) standard length (SL), large helpers 4.01 ± 0.08 cm, breeder females 5.52 ± 0.10 cm and breeder males 6.72 ± 0.20 cm. In experiment 2, small helpers measured 2.98 ± 0.06 cm, large helpers 3.92 ± 0.05 cm, breeder females 5.57 ± 0.09 cm and breeder males 6.80 ± 0.10 cm. The sexes of the large helpers were equally balanced across groups within both experiments. Sexes were determined by inspection of the genital papillae. The smaller helpers were immature at the beginning of the experiments and could not be sexed because genital papillae were not yet differentiated. In Experiment 1, the small helpers reached sexual maturity during the experiment and were sexed at the end of the trials (three males, two females). In Experiment 2, this was not possible because the small helpers did not reach sexual maturity during the experiment. Tanks of 200 L were divided in two 100-L compartments by water-tight opaque partitions. Each group was housed in a 100-L compartment equipped with a 2-cm layer of fine-grained sand on the bottom, a biological filter, two flower pot halves serving as shelters, and an opaque PVC tube and a semi-transparent plastic bottle mounted near the water surface as additional shelter (Fig. S1a).

For Experiment 2 additionally five small (SL: 2.79 ± 0.05 cm) and five large (SL: 3.68 ± 0.04 cm) juvenile *N. pulcher* and seven specimens of the egg predator *Telmatochromis vittatus* (SL: 4.46 ± 0.16) were used. Each individual was used in multiple trials. Between trials, the additional *N. pulcher* were kept in two 100-L tanks (five fish per tank), while the *T. vittatus* were returned to conspecific aggregations in their home

tanks.

All tanks were kept on a 13:11 light:dark cycle and at a water temperature of 27 ± 1 °C, simulating the conditions in Lake Tanganyika (Arnold and Taborsky, 2010). All fish were fed once per day with commercial food flakes (5 days/week) or frozen zooplankton (1 day/week).

2.3. Function of 5-HT 1A receptor, and its agonist and antagonist

The 5-HT_{1A} receptor is one of the most abundant receptors of the serotonergic system in mammals. Molecular analysis showed that in fish the amino acid sequence of this receptor is very similar to its homologue in humans (Medeiros and McDonald, 2013). Like all 5-HT_1 receptors it is characterized by its high affinity for serotonin (Nichols and Nichols, 2008). The 5-HT_{1A} receptors are present both as presynaptic autoreceptors and as postsynaptic heteroreceptors (Hannon and Hoyer, 2008). Two drugs have been successfully used in fish before, which act as agonist and antagonist on the 5-HT_{1A} receptor, respectively (Clotfelter et al., 2007; Paula et al., 2015): 8-OH-DPAT (7-(Dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol) and Way-100635 (N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]-ethyl]-N-(2-pyridyl)cyclohexanecarboxamide). An activation of the receptor by binding to either serotonin or the agonist 8-OH-DPAT inhibits the adenylyl cyclase activity and opens potassium channels and thus causes neuronal hyperpolarization leading to a reduction of the firing rate. If serotonin or an agonist binds to the $5HT_{1A}$ autoreceptor (i.e. pre-synaptic) it leads to a hyperpolarization of 5-HT neurons (Lanfumey and Hamon, 2000) in the nucleus raphe areas (Barnes and Sharp, 1999), leading to a suppression of 5-HT synthesis, turnover and release (Frazer and Hensler, 1999; Nichols and Nichols, 2008). When it binds post-synaptically, it causes a decrease in the firing rate of the postsynaptic cells mostly located in cortical and limbic areas (Barnes and Sharp, 1999). 8-OH-DPAT binds preferably at the presynaptic autoreceptor, and only at higher doses it also binds to the postsynaptic heteroreceptor (Hjorth and Magnusson, 1988; Zhao et al., 2019). Correspondingly, the application of the highly selective 5-HT_{1A} antagonist Way-100635 (rev. in Forster et al., 1995) causes continued firing pre- and post-synaptically. It can fully reverse effects of 8-OH-DPAT on anxiety (Collinson and Dawson, 1997) and cognitive impairment (Helsley et al., 1998).

2.4. Drugs

Way-100635 and 8-OH-DPAT (product numbers W108 and H8520, Sigma Aldrich, Deisenhofen, Germany) were dissolved in saline solution (0.9% NaCl) to the respective concentration. In Experiment 1 three different concentrations of the two drugs were tested (8-OH-DPAT: 0.5, 1.0 and 2.0 μg/gbw (gbw = gram body weight); WAY-100635: 0.5, 1.5 and 3.0 μg/gbw. These concentrations were chosen based on previous studies (Clotfelter et al., 2007; de Boer et al., 1999; Lopez-Mendoza et al., 1998; Paula et al., 2015; Sperry et al., 2003). For Experiment 2 only one concentration of each drug was applied (see 'Results'). Directly after preparation and whenever they were not used the drug-solutions were stored at −20 °C.

2.5. Injections

We injected the respective focal fish of a trial with either the 5-HT_{1A} receptor agonist 8-OH-DPAT or the antagonist Way-100635. A standardized amount of 15 μL/gbw of drug solution were injected into the tail muscle after the respective focal fish had been anesthetized with KOI MED® Sleep (Koi & Bonsai Zimmermann, Bühlertann, Germany). The injections were performed using a 0.5 mL insulin syringe for the large helpers and a 25 μL Hamilton® syringe with one-way needles for the small helpers. The Hamilton® syringes were disinfected and cleaned with acetone and distilled water between injections. After an injection the fish was placed in a plastic box, filled with water aerated by an airstone, for 5 min for recovery from anaesthesia. Afterwards it was placed into a small mesh cage within in its home tank for another 5 min of recovery, during which it fully regained its normal swimming activity, before it was released into its home tank.

2.6. Experiment 1: Dosage-dependence

We determined concentrations and observation intervals after injection of 8-OH-DPAT and Way-100635, which had the strongest behavioural effects. In this experiment 10 helper individuals of five social groups, one at a time, were injected with the three concentrations of 8-OH-DPAT, the three concentrations of WAY-100635 and the control solution in seven successive trials. The behaviour was recorded for 10 min directly before each injection and at four time-intervals (15 min, 30 min, 45 min and 60 min) after the injection, again for 10 min each. The order of treatments was balanced, and each focal fish had a gap of at least three days between consecutive injections to ensure independence of the trials. Way-100635 and 8-OH-DPAT are known to be degraded quickly, namely they have a terminal elimination half-life of 33 min (Way-100635) and 143 min (8-OH-DPAT), respectively, in rats (Zuideveld et al., 2002). The social groups received a break of at least 23 h between successive trials. The behavioural observations and injections were performed between 09:00 h 15:00 h. The time window was kept as small as possible to minimise effects of potential diurnal variation in serotonin activity (Fingerman, 1976) that might influence the effect of the treatment and thus the behavioural response.

2.7. Experiment 2: Context-dependence

We recorded how receptor agonist and antagonist affected social interactions with other group members and territorial intruders. The intruder challenges consisted of (i) a conspecific of similar size as the focal fish, which should elicit aggressive behaviour by the focal fish, because it challenges the latter's position in the hierarchy; and (ii) the egg predator *Telmatochromis vittatus*, which predates on the egg and larvae produced by the dominant breeders of the groups. In nature egg predators are jointly attacked by all group members, but in particular by subordinate helpers.

In this experiment, 24 helpers of 12 social groups successively received three injections, one per drug, with the concentrations that elicited the strongest behavioural response as determined in Experiment 1 (i.e., 1.0 μg/gbw for 8-OH-DPAT and 1.5 μg/gbw for WAY-100635; see 'Results') and saline solution as control. Gaps between successive injections were at least 3 days. We recorded the social behaviour in the group 10 min before each injection. Thirty min after the injections, the spontaneous behaviour of the group was recorded for another 10 min. At 45 and 60 min after the injections, respectively, intruder tasks together with further 10 min behavioural recordings were performed. For the conspecific intruder task, size-matched juvenile *N. pulcher* (max. ±0.25 cm difference to focal fish) was used; for the egg predator intruder task, we used adult *T. vittatus*. Intruder individuals were randomly captured from stock tanks of the two species and transferred to a clear Plexiglas tube of 10.5 cm diameter (length 15.5 cm), equipped with some sand on the ground and closed with a mesh lid, allowing smell to diffuse. One min before the start of the 10-min behavioural recording, the tubes with the respective intruder individuals were placed in the front corner of the group compartment (see Fig. S1b). After the 10-min recording, the tube with the intruder was removed from the group tank and the respective intruder was placed back in its holding tank. In each trial both intruder species were used successively. The order of conspecific and egg predator intruder task was balanced between the different helper individuals but was kept the same for successive trials of the same individual.

2.8. Behavioural recordings

All behavioural recordings were done by live observations of

behaviours within the home tanks of fish using the software 'Observer' version 5.0.25 (Noldus, the Netherlands, 2003). The observer (PS) was blind to the treatment while recording the behaviour. As backup, all trials were additionally video-recorded (Experiment 1: Sony DCR-SR200; Experiment 2: Sony HDR-CX550). The following behaviours were recorded: Overt aggression (bite, ram, mouthfight), restrained aggression (fin spread, head down, opercula spread, approach/chasing), affiliative behaviour (bumping, following, swimming towards), submissive behaviour (tail quiver, hook display), and escape/evade (for descriptions of behaviours see Table S1). Additionally, the time the focal fish spent in one of the shelters (flower pot, PVC tube, bottle or behind filter) was recorded. During the behavioural recording before the injection, the acceptance of the focal fish in the group was assessed at three levels (see Fischer et al., 2017); Level 1: The focal fish was tolerated as long as it stayed near a corner of the tank, but it was attacked as soon as it approached the centre; Level 2: The focal fish was accepted in the centre, but was not allowed to enter the shelter; Level 3: The focal fish was accepted everywhere in the tank.

In both experiments we assessed the activity of the focal fish. In Experiment 1, the videos from 30 and 45 min after the injection with the intermediate concentrations of the agonist and the antagonist (1 μg/gbw 8-OH-DPAT, 1.5 μg/gbw Way-100635), respectively, were analysed for activity, as those are the time points and concentrations for which effects on behaviours were most pronounced. Every 30 s during a recording, we noted whether the focal fish was hiding or staying near a hiding place, respectively (activity: 'hiding'). When the fish was not hiding, we noted whether it was moving (activity: 'swimming') or not. In Experiment 2, the same measures of activity were taken, but we recorded them during the live observations and at all four time points of each trial. The activity of the conspecific intruder and the egg predator (*T. vittatus*) within the presentation tubes was recorded from the videos taken of the egg predators during the trials. We noted every 30 s if intruders were swimming or standing still.

2.9. Data analysis

All statistical analyses were done with the statistical software R, version 3.4.2 (R Core Team, 2017). For the behavioural count data of Experiment 1, we fitted negative binomial general linear mixed-effect models (GLMMs) using the package 'lme4' (Bates et al., 2015). A separate model was fitted for each time point. All occurrences of restrained and overt aggression were summed up and analysed as total aggression. Tail quiver and hook display were summed up as measure for submission. All affiliative behaviours were summed up and analysed as one variable. Treatment (i.e. the drug injected), focal fish (small or large helper), sex and acceptance level were included as fixed factors. Group identity was included as random factor. To control for model convergence, we used the optimizer 'bobyqa'. For those drug concentrations for which focal fish activity has been measured (8-OH-DPAT 1 μg/gbw and Way-100635 1.5 μg/gbw), we fitted one model for each behavioural category (aggression, submission, affiliation) using the data of both time points with activity measures (30 and 45 min) and included treatment, swimming activity, time point and acceptance level (see above) as fixed factors and identity of focal fish as random factor. In these three models, only trials were included, in which during the measurement of activity state every 30 s (see above) a focal fish was at least five times outside of the hiding places. One biological outlier was removed, as one focal fish showed no submission at all despite being frequently attacked.

For the Experiment 2, we compiled two separate datasets for the two drugs. We fitted GLMMs with Poisson distribution or negative binomial distribution (indicated as 'nbGLMM' in the 'Results') for all behavioural counts, using the package 'lme4' (Bates et al., 2015) and the optimizer 'bobyqa' to control for model convergence. We merged the behaviours in the same way as in Experiment 1, and analysed the variables 'total aggression', 'submission' and 'affiliation'. Treatment, intruder species, the order of intruder presentation, standard length and sex of the focal fish, the acceptance level, and activity (with three levels: standing still, swimming, hiding) were included as fixed factors. Group identity and identity of focal fish were included as random factors. All Poisson GLMM models were checked for overdispersion, which never occurred. AIC-based model selection was used to simplify the model. To that end, we performed a stepwise backward selection (Zuur et al., 2009) starting with the full model and always removing the variable that maximally reduced the AIC. We stopped simplifying the model when the deletion of a variable would not lead to an AIC that is at least 2.0 smaller than the one of the previous model (see Symonds and Moussalli, 2011).

3. Results

3.1. Experiment 1: Dosage-dependence

Aggression: Both drugs reduced aggressive behaviour towards group members in this experiment. Compared to the control injection with saline solution, total aggression was significantly reduced 30 min after injection for 1 μg/gbw of 8-OH-DPAT ($P = 0.049$) and there was a trend in the same direction for 1.5 μg/gbw of Way-100635 ($P = 0.055$) (nbGLMM, Table S2b, Fig. 1B). Aggressive behaviour still tended to be reduced 45 min after injection of the same drugs as compared to the control injection (1 μg/gbw 8-OH-DPAT: $P = 0.056$, 1.5 μg/gbw Way-100635: $P = 0.096$; nbGLMM, Table S2c, Fig. 1C). Finally, also 60 min after the injection 8-OH-DPAT 1 μg/gbw still tended to attenuate aggression ($P = 0.057$; nbGLMM, Table S2d, Fig. 1D). Also, the highest concentration of the agonist (2 μg/gbw of 8-OH-DPAT) significantly reduced total aggression compared to saline solution ($P = 0.004$; nbGLMM, Table S2d, Fig. 1D). In the model that was corrected for activity using only the data for 30 and 45 min after the injection (see 'Data analysis') of 1 μg/gbw for 8-OH-DPAT and 1.5 μg/gbw for Way-100635, there was only a significant negative effect of the antagonist on aggressive behaviour compared to saline solution ($P = 0.017$; nbGLMM, Table S2e).

3.1.1. Submission

The injection of 1 μg/gbw of 8-OH-DPAT induced a significant decrease of submissive behaviour towards other group members, 15 min ($P = 0.047$; nbGLMM, Table S3a, Fig. 2A) and 30 min after injection ($P = 0.001$; nbGLMM, Table S3b, Fig. 2B) compared to the injection of saline solution. At 30 min, also the lowest concentration of 8-OH-DPAT (0.5 μg/gbw) tended to decrease submission compared to saline ($P = 0.069$; nbGLMM, Table S3b, Fig. 2B). The two higher concentrations of 8-OH-DPAT significantly downregulated submission 45 min (1 μg/gbw: $P = 0.007$, 2 μg/gbw: $P = 0.007$; nbGLMM, Table S3c, Fig. 2C) and 60 min after injection (1 μg/gbw: $P = 0.032$, 2 μg/gbw: $P = 0.007$; nbGLMM, Table S3d, Fig. 2D) compared to the control. The model controlling for the effect of activity (intermediate concentrations of both drugs measured at 30 and 45 min after injection only) revealed a negative effect of 8-OH-DPAT on submission ($P = 0.010$, nbGLMM, Table S3e) compared to saline solution. Way-100635 did not influence submission significantly at any of the three concentrations.

3.1.2. Affiliative behaviour

Thirty min after the injection the two lower concentrations of the agonist 8-OH-DPAT had a significant negative effect on affiliation, and the highest concentration tended to reduce affiliative behaviour compared to the control (0.5 μg/gbw: $P = 0.020$, 1.0 μg/gbw: $P = 0.016$, 2.0 μg/gbw: $P = 0.062$; nbGLMM, Table S4b, Fig. 3B). All concentrations of 8-OH-DPAT significantly reduced affiliation after 45 min compared to saline solution (0.5 μg/gbw: $P = 0.045$, 1.0 μg/gbw: $P = 0.010$, 2.0 μg/gbw: $P = 0.003$; nbGLMM, Table S4c, Fig. 3C). After 60 min, the two higher concentrations of 8-OH-DPAT still had a significant negative effect on affiliative behaviour compared to the saline solution (1.0 μg/gbw: $P = 0.008$, 2.0 μg/gbw: $P < 0.001$; nbGLMM, Table S4d, Fig. 3D). The model corrected for activity (intermediate concentrations only, data

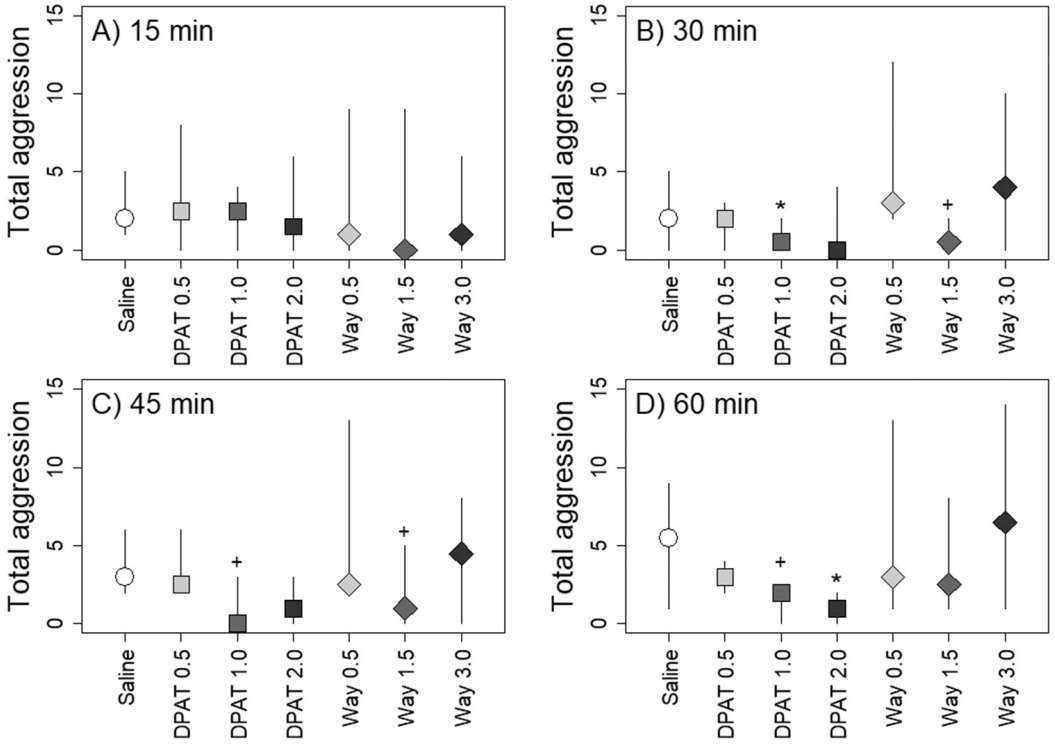

Fig. 1. The effect of different concentrations of 8-OH-DPAT and Way-100635 on the frequency of total aggression shown during 10 min in Experiment 1 at four time points after the injection: (A) 15–25 min, (b) 30–40 min, (c) 45–55 min, and (d) 60–70 min. Medians and quartiles of raw data are shown. An asterisk indicates a significant effect ($p < 0.05$) and a plus sign indicates a trend ($0.05 < p < 0.1$) compared to saline injection.

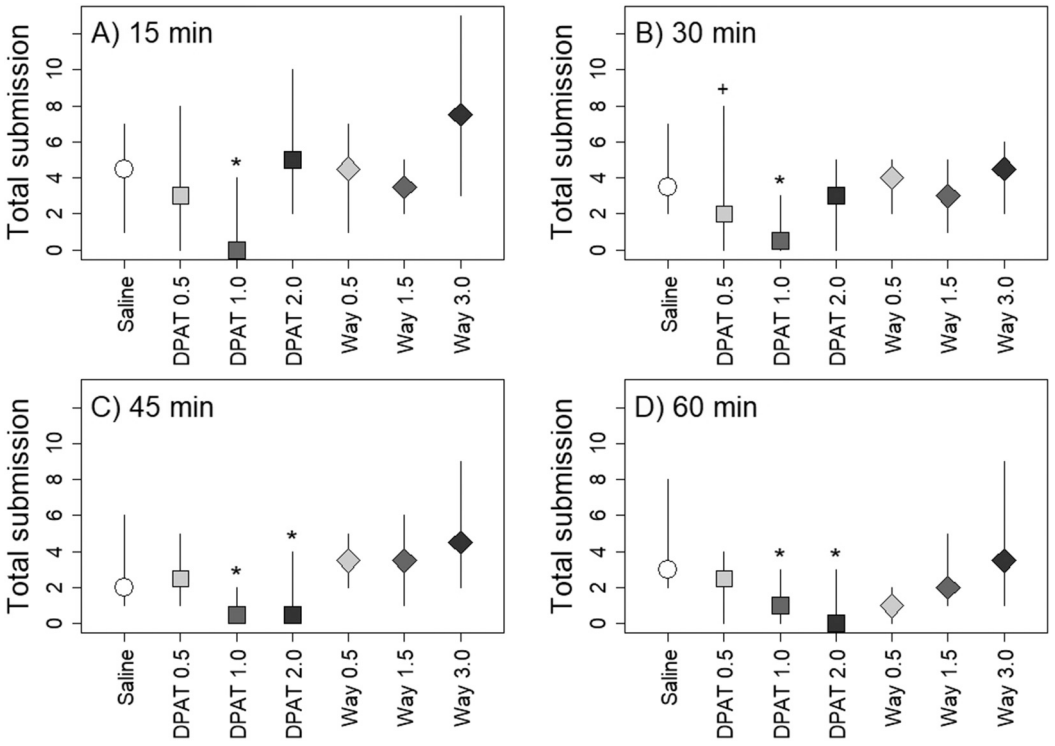

Fig. 2. The effect of different concentrations of 8-OH-DPAT and Way-100635 on the frequency of submission shown during 10 min in Experiment 1 at four time points after the injection: (A) 15–25 min, (b) 30–40 min, (c) 45–55 min, and (d) 60–70 min. Medians and quartiles of raw data are shown. An asterisk indicates a significant effect ($p < 0.05$) and a plus sign indicates a trend ($0.05 < p < 0.1$) compared to saline injection.

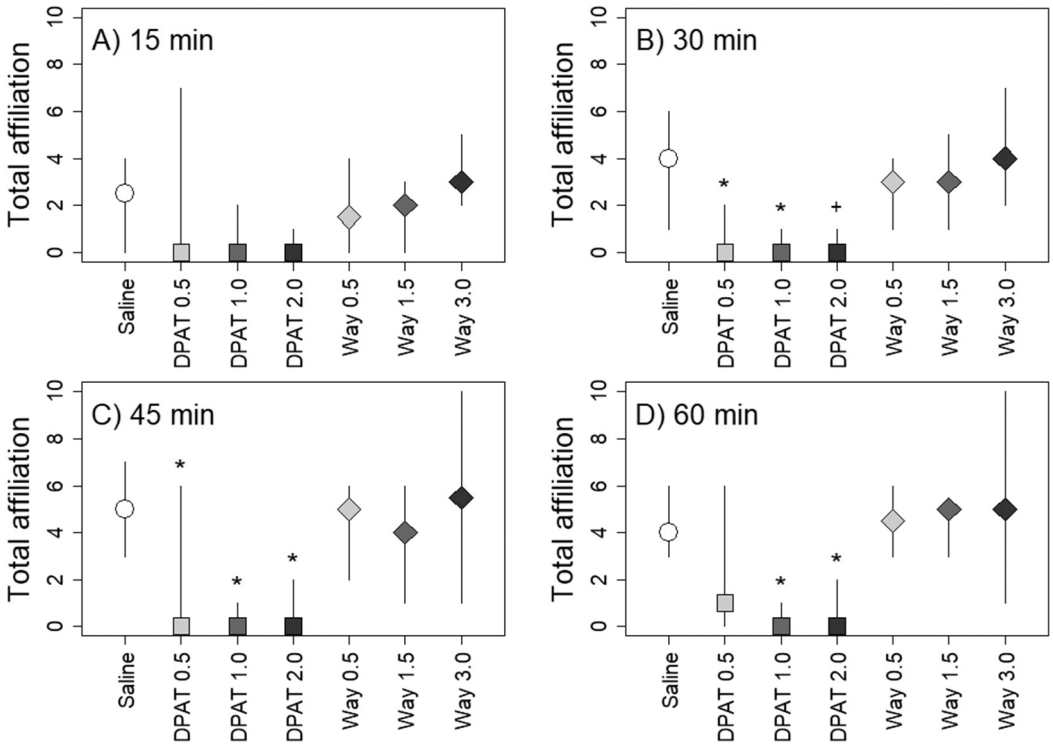

Fig. 3. The effect of different concentrations of 8-OH-DPAT and Way-100635 on the frequency of affiliation shown during 10 min in Experiment 1 at four time points after the injection: (A) 15–25 min, (b) 30–40 min, (c) 45–55 min, and (d) 60–70 min. Medians and quartiles of raw data are shown. An asterisk indicates a significant effect ($p < 0.05$) and a plus sign indicates a trend ($0.05 < p < 0.1$) compared to saline injection.

of 30 and 45 min past injection) revealed no significant effect of the drugs on affiliative behaviour. Way-100635 did not influence affiliative behaviour significantly at any of the three concentrations.

3.2. Experiment 2: Context-dependence

3.2.1. Behaviour towards intruders

We only analysed aggressive behaviour towards the intruders during the challenge tests, because no socio-positive behaviour is directed towards intruders. There were no interactions between presence or absence of drugs and the two intruder types (non-significant interaction terms removed from the final models; section 'Data analysis'). The drugs also had no main effects on aggression towards the intruders. The focal fish showed significantly less aggression towards the egg predator than towards the conspecific intruder in the trials with 8-OH-DPAT ($P <$ 0.001; GLMM, Table S6a, Fig. 4a), whereas there was a trend in the same direction in the trial with Way-100635 ($P = 0.075$; GLMM, Table S6b, Fig. 4b).

3.2.2. Behaviour towards group members

3.2.2.1. Aggression. There was no significant interaction between the type of trials (control, conspecific or egg predator) and whether a drug (8-OH-DPAT or Way-100635) or saline was injected (non-significant interaction terms removed from the final models; section 'Data analysis'). Across the three types of trials the agonist 8-OH-DPAT significantly increased aggressive behaviour of the focal fish towards other group members compared to saline solution ($P = 0.027$; GLMM, Table S7a, Fig. 5A). Overall, the antagonist did not affect aggressive behaviour towards group members (Table S7b). In the presence of a

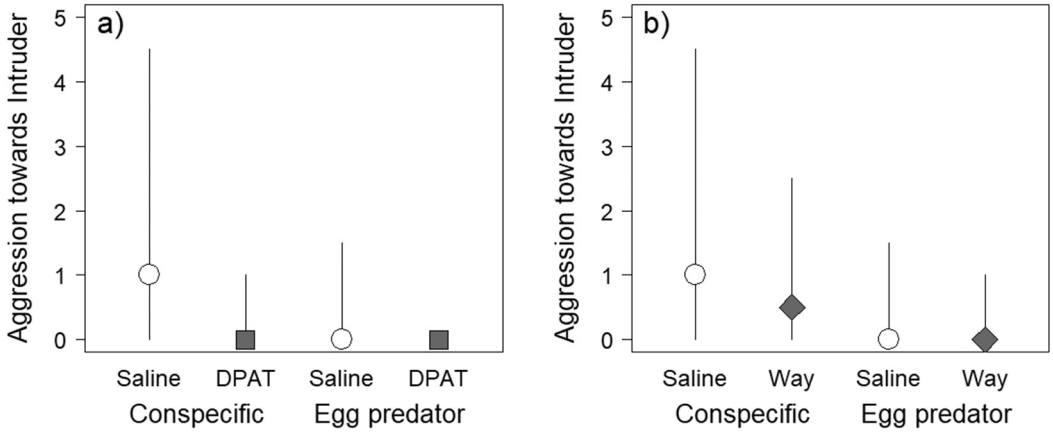

Fig. 4. The effect of (a) 8-OH-DPAT and (b) Way-100635 on aggression towards a conspecific intruder and an egg predator intruder, respectively, in Experiment 2. Medians and quartiles of raw data are shown.

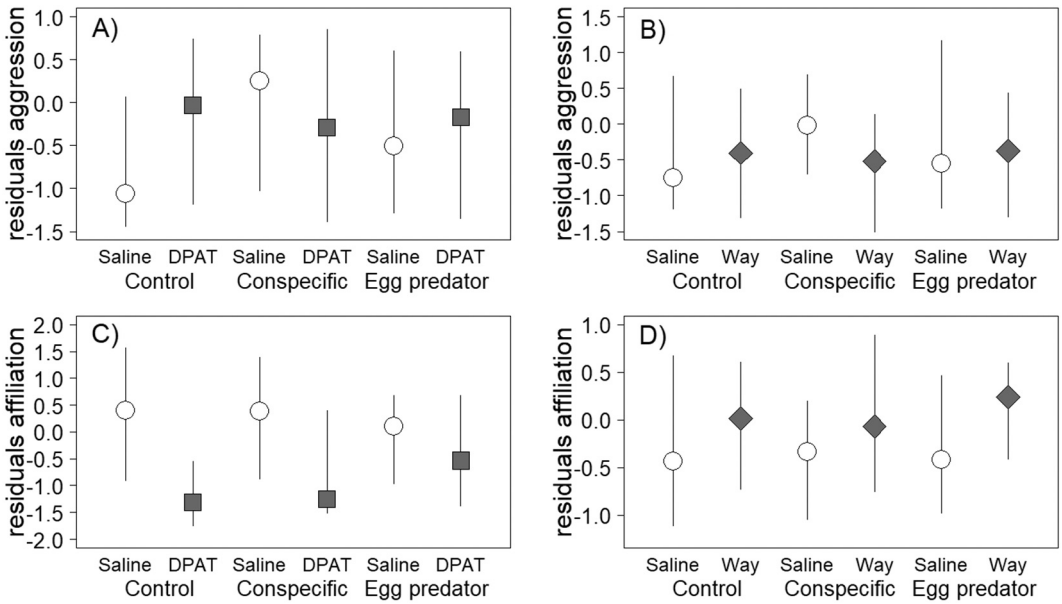

Fig. 5. The effect of (A,C) 8-OH-DPAT and (B,D) Way-100635 in Experiment 2 on (A,B) aggression and (C,D) affiliation towards other group members in the control situation, when a conspecific intruder was presented and when an egg predator intruder was presented. Medians and quartiles are shown of model residuals correcting for the variation in all model factors except drug treatment.

conspecific intruder the focal fish increased their aggression towards other group members compared to trials without intruder, irrespective of the drug (8-OH-DPAT: $P < 0.001$, GLMM, Table S7a, Fig. 4A; Way-100635: $P < 0.001$, GLMM, Table S7b, Fig. 5B). If an egg predator was presented, focal fish only tended to increase their aggression towards the other group members (8-OH-DPAT: $P = 0.076$, GLMM, Table S7a, Fig. 5A; Way-100635: $P = 0.068$, GLMM, Table S7b, Fig. 5B).

3.2.2.2. Submission. The interaction term between type of trial and whether a drug or saline was injected was not-significant and hence removed from the final model. Moreover, none of the drugs influenced submissive behaviour as main effect. In the model testing for effects of the antagonist Way-100635, the focal tended to show less submission towards group members if either of the intruders were present compared to when intruders were absent (conspecific: $P = 0.061$, egg predator: $P = 0.074$; GLMM, Table S8b), both in the saline and the antagonist treatment. In model testing for effects of 8-OH-DPAT, none of the intruders influenced the amount of submission shown towards group members.

3.2.2.3. Affiliation. There was a significant interaction between the presence or absence of 8-OH-DPAT and the presence or absence of an egg predator ($P = 0.013$; GLMM, Table S9a, Fig. 5C). Inspection of Fig. 5C suggests that this interaction was caused by a stronger negative influence of the agonist on affiliative behaviour in the control trial than during the egg predator presentation. We reran this GLMM after removing the significant interaction to be able to interpret the results of the main effects of 8-OH-DPAT and intruder species. The effects on all main effects hardly changed after removal of the interaction (Table S9a). Affiliative behaviour was significantly decreased after an injection of 8-OH-DPAT compared to the control injection ($P < 0.001$; GLMM, Table S9a, Fig. 5C) across all intruder treatments. Conversely, injection of Way-100635 significantly increased affiliation compared to the control ($P = 0.023$; GLMM, Table S9b, Fig. 5D) across all challenges. For both drugs, affiliation was lower if a conspecific intruder was presented compared to the control without intruder (8-OH-DPAT: $P = 0.016$, GLMM, Table S9a, Fig. 5C; Way 100635: $P = 0.009$; GLMM, Table S9b, Fig. 5D).

4. Discussion

Here we performed a two-step experimental study to investigate the modulating effect of the 5-HT_{1A} receptor on aggressive and sociopositive behaviours in a highly social cichlid fish. In the dosage-dependent experiment, the 5-HT_{1A} receptor agonist 8-OH-DPAT reduced the amount of submissive displays and the antagonist Way-100635 reduced aggression. In the context-dependent experiment applying the agonist increased aggression and decreased affiliative behaviour, whereas the antagonist increased affiliative behaviour. Our study shows how 5-HT_{1A} is involved in regulating aggressive and affiliative behaviour within the social context of group living.

4.1. Experiment 1: Dosage-dependence

We first aimed to obtain a species-specific dosage-response-curve for the used drugs and to find the optimal timespan after the injections to record behaviour. Fifteen min after the injection the drugs had hardly any effect, whereas 30 to 60 min after injection, 8-OH-DPAT inhibited all social behaviours, particularly at the intermediate concentration of 1.0 μg/gbw. Instead, Way-100635 tended to reduce aggression at the intermediate concentration of 1.5 μg/gbw at 30 and 45 min post-injection, whereas it had no effect on socio-positive behaviour.

Because, the application of 8-OH-DPAT reduced all social behaviours, we hypothesised that this drug may have reduced either the locomotory activity of our experimental animal, as it had been shown for serotonin (e.g. Gerson and Baldessarini, 1980) and 8-OH-DPAT (Olivier et al., 1995), or their general social motivation (Olivier et al., 1995). Clearly this drug has a very strong motor inhibitory effect at high doses, as we observed in some trials focal fish swimming passively near the water surface (see also Olivier et al., 1995; Stewart et al., 2013). This surfacing behaviour might be part of a serotonin syndrome, which is an adverse condition implied by an exaggerated serotonergic activity. In humans it is characterized by symptoms like fever, confusion and ataxia and can be life-threatening (Sporer, 1995).

As changes in locomotor activity may alter the probability to encounter and engage with group members, it is necessary to disentangle the effects of activity from those of the drugs in regulating the analysed behaviours. Therefore, we tested whether controlling for

locomotory activity in our statistical analyses would explain some of the drug effects on social behaviour. To avoid multiple testing, we did this second analysis only for two time points (30 and 45 min) and only for the intermediate concentration, where the drugs had the highest effects. Correcting for activity changed the results in two important ways. (i) 8-OH-DPAT only reduced submission for the intermediate dose of 1.0 μg/gbw. (ii) The intermediate dose of 1.5 μg/gbw Way-100635 significantly decreased aggression. All other effects found before the correction for activity (Table S5) had vanished. The significant effects on social behaviour after correcting for locomotory activity are opposite to predictions based on previous studies. In *B. splendens* activating the 5-HT_{1A} receptor by 8-OH-DPAT down-regulated aggression (Clotfelter et al., 2007), and *A. carolensis* increased submission (Deckel, 1996), whereas we found a decrease in submission. Applying Way-100635 reduced aggression, whereas Clotfelter et al. (2007) did not find effects on aggression.

We propose that these differences might be related to the different social contexts of experimental tests. While we measured spontaneous aggressive behaviour, in *B. splendens* aggression was directed towards the own mirror image in a simulated contest (Clotfelter et al., 2007). However, mirror fights are by definition always unresolved and lead to different monoaminergic concentrations in the brain as compared to real contests (Teles et al., 2013). An alternative or additional, possible reason for the contradicting results is a context specificity with respect to the stress level the test fish were exposed to. Stress and serotonergic action are known to interact, and the 5-HT_{1A} receptor is involved in this interaction (reviewed in: Chaouloff et al., 1999; Puglisi-Allegra and Andolina, 2015). While there were no signs of stress in the focal fish in our experiment and the focal fish were tested in the relaxed context of their social group and home tank, *B. splendens* (Clotfelter et al., 2007) and *A. carolensis* (Deckel, 1996) were tested in a highly aggressive and thus most likely very stressful contest situations.

4.2. Experiment 2: Context-dependence

4.2.1. Behaviour towards intruders

By challenging the focal fish by live-presentations of two types of intruders at time points 45 and 60 post-injection we explored context-specific effects of the 5-HT_{1A} receptor on aggressive behaviour. We had predicted an interaction between 5-HT_{1A} activity and intruder challenges. In particular, we had predicted that the drugs modulate aggressive responses differently in conspecific competitors than in egg predators, because the former directly challenges the hierarchy position of a focal fish, whereas the latter is a threat only to offspring produced by the dominant pair. While indeed there was a main effect showing that egg predators were attacked less than conspecific intruders (Table S6a, Fig. 4), aggression towards the intruders was not interactively affected by the drugs and intruder type (non-significant interaction has been removed from the model).

4.2.2. Behaviour towards other group members

There was a significant interaction of drug (saline or agonist) by intruder type affecting affiliative behaviour towards the other group members. It was caused by the difference between saline and drug being larger in the absence of intruders as compared to when intruders were present (cf. Fig. 5c). We had predicted this interaction, because a sociopositive behaviour such as affiliation is of greatest importance, when subordinate group members have no opportunities to appease the dominants by helping (Bergmüller and Taborsky, 2005; Fischer et al., 2017). So the effect of drugs modulating affiliation should be strongest in the absence of possibilities to show helping behaviour. There were no statistical interactions between saline and drugs by intruder type for the other social behaviours.

The drugs had several main effects on social behaviours. As predicted, affiliation was affected in the opposite direction than aggression by the drugs. Affiliation and aggression are two behaviours with entirely contrasting intentions and effects, and thus are unlikely to be shown simultaneously by an individual (Kelly and Vitousek, 2017). The focal fish decreased affiliative behaviour towards other group members when treated with the agonist 8-OH-DPAT in accordance with previous findings in primates (Larke et al., 2016), and accordingly they increased affiliative behaviour after injection with the antagonist Way-100635, irrespective of the type of challenge. Thus, our results show that 5-HT_{1A} activity regulates affiliative behaviour in *N. pulcher*.

Focal fish showed more aggressive behaviour towards other group members when treated with the agonist 8-OH-DPAT, which differs from the findings of Experiment 1, where after controlling for activity there was no significant effect of the agonist. The decrease of aggressive behaviour typically observed after treatment with the agonist is thought to be modulated through the presynaptic serotonin 1A autoreceptors (de Boer, 2000). On the contrary the serotonin syndrome observed after high doses of agonists is known to be regulated via the postsynaptic 5-HT_{1A} receptors (Lucki, 1992; Tricklebank et al., 1984). Previous findings show that 8-OH-DPAT preferably binds to 5-HT_{1A} autoreceptors, and that higher doses (1.0 μg/gbw) are needed for it to bind also postsynaptically (Hjorth and Magnusson, 1988; Zhao et al., 2019). Thus, we might have triggered both the presynaptic autoreceptor and also the postsynaptic receptor in this experiment. Binding presynaptically only vs both pre- and postsynaptically has led to opposite behavioural phenotypes in a different behavioural context, i.e. anxiety behaviour (File et al., 1996; rev. in Garcia-Garcia et al., 2014). The possible reason, why our treatment may have triggered pre- and postsynaptic receptors in Experiment 2 resulting in increased aggression towards group members may be related to the presence of intruders in this experiment. The presence of intruders generally increased aggression of focal fish towards other group members (Table S6a), which might represent a carry-over effect of the aggression directed towards the intruders. So although this is still speculative, the presence of intruders may have interacted with the strength of the effect of the agonist on aggressive behaviour.

4.2.3. Drug effects in experiment 1 vs. experiment 2

Interestingly the two experiments revealed effects on different behaviours thereby complementing each other. In Experiment 1, 8-OH-DPAT decreased submissive behaviour towards group members, whereas in Experiment 2 affiliative behaviour was decreased by the agonist, and it was increased by the antagonist. Moreover, the agonist increased aggression in Experiment 2, and the antagonist decreased aggression in Experiment 1. Taken together, the two drugs affected the aggressive and sociopositive behaviours in opposite directions highlighting serotonin 1A receptor as an important player in the modulation of social behaviour (Table S10).

4.3. General discussion

It is increasingly acknowledged that the function of brain mechanisms involved in the regulation of behaviour should account for the natural history of study subjects by testing them in experimental settings that are ecologically relevant (Hofmann et al., 2014). In particular, it is important to test the function of brain mechanisms in different social and ecological contexts. Pharmacological manipulation of the serotonin system provide a good example for this: By testing the role of the 5-HT_{1A} receptor in a cooperative context (Paula et al., 2015), in pair-bonding (Larke et al., 2016), or in stable social groups (this study) additional functions of this receptor for the expression of social behaviours have been detected beyond its effect on aggressive behaviours previously described in several vertebrates. From our results, we can conclude that the serotonin 1A receptor does play an important role not only in the modulation of aggressive, but also of submissive and affiliative behaviour.

Context-specificity of the effects of 5-HT_{1A} receptor agonist and antagonist on social behaviour might also explain deviations from previous findings reported in this study. One possible mechanism

contributing to context-specificity and the different outcomes of studies concerns differences in stress levels caused by the testing set-ups. Endocrinological states, such as stress levels, linked to environmental stressors might influence the role of serotonin in behavioural regulation. We hypothesize that under low stress levels as they were present in this study serotonin decreases submission and increases aggression, whereas under high stress levels as they may have pertained to aggressive contest situations serotonin increases submission and decreases aggression. This hypothesis needs further testing, in particular, as interactions between cortisol and the 5-HT_{1A} receptor have been reported, which would need to be taken into account (reviewed in: Chaouloff et al., 1999; Puglisi-Allegra and Andolina, 2015).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2020.104918.

References

Arnold, C., Taborsky, B., 2010. Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids. Anim. Behav. 79, 621–630. https://doi.org/10.1016/j.anbehav.2009.12.008.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152. https://doi.org/10.1016/S0028-3908(99)00010-6.

Barosky, A.-L., Taylor, J., Massari, V.J., 1983. Dorsal raphe-hypothalamic projections provide the stimulatory serotonergic input to suckling-induced prolactin release. Endocrinology 113, 1894–1903. https://doi.org/10.1210/endo-113-5-1894.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Soft. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Bergmüller, R., Taborsky, M., 2005. Experimental manipulation of helping in a cooperative breeder: helpers "pay to stay" by pre-emptive appeasement. Anim. Behav. 69, 19–28. https://doi.org/10.1016/j.anbehav.2004.05.009.

Chaouloff, F., Berton, O., Mormède, P., 1999. Serotonin and stress. Neuropsychopharmacology 21, 28S–32S. https://doi.org/10.1016/j.bbr.2014.07.052.

Clotfelter, E.D., O'Hare, E.P., McNitt, M.M., Carpenter, R.E., Summers, C.H., 2007. Serotonin decreases aggression via 5-HT1A receptors in the fighting fish *Betta splendens*. Pharmacol. Biochem. Behav. 87, 222–231. https://doi.org/10.1016/j.pbb.2007.04.018.

Collinson, N., Dawson, G.R., 1997. On the elevated plus-maze the anxiolytic-like effects of the 5-HT 1A agonist, 8-OH-DPAT, but not the anxiogenic-like effects of the 5-HT 1A partial agonist, buspirone, are blocked by the 5-HT 1A antagonist, WAY 100635. Psychopharmacology 132, 35–43. https://doi.org/10.1007/s002130050317.

de Boer, S., 2000. Somatodendritic 5-HT1A autoreceptors mediate the anti-aggressive actions of 5-HT1A receptor agonists in rats an ethopharmacological study with S-15535, alnespirone, and WAY-100635. Neuropsychopharmacology 23, 20–33. https://doi.org/10.1016/S0893-133X(00)00092-0.

de Boer, S.F., Lesourd, M., Mocaer, E., Koolhaas, J.M., 1999. Selective antiaggressive effects of alnespirone in resident-intruder test are mediated via 5-hydroxytryptamine1A receptors: a comparative pharmacological study with 8-hydroxy-2-dipropylaminotetralin, ipsapirone, buspirone, eltoprazine, and WAY-100635. J. Pharmacol. Exp. Ther. 288, 1125–1133.

Deckel, A., 1996. Behavioral changes in *Anolis carolinensis* following injection with fluoxetine. Behav. Brain Res. 78, 175–182.

Edwards, D.H., Kravitz, E.A., 1997. Serotonin, social status and aggression. Curr. Op. Neurobiol. 7, 812–819. https://doi.org/10.1016/S0959-4388(97)80140-7.

File, S.E., Gonzalez, L.E., Andrews, N., 1996. Comparative study of pre- and postsynaptic 5-HT(1A) receptor modulation of anxiety in two ethological animal tests. J. Neurosci. 16, 4810–4815. https://doi.org/10.1523/jneurosci.16-15-04810.1996.

Fingerman, S.W., 1976. Circadian rhythms of brain 5-hydroxytryptamine and swimming activity in the teleost, *Fundulus grandis*. Comp. Biochem. Physiol. Part C. Comp. 54, 49–53. https://doi.org/10.1016/0306-4492(76)90024-1.

Fischer, S., Zöttl, M., Groenewoud, F., Taborsky, B., 2014. Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. Proc. R. Soc. B Biol. Sci. 281, 20140184. https://doi.org/10.1098/rspb.2014.0184.

Fischer, S., Bohn, L., Oberhummer, E., Nyman, C., Taborsky, B., 2017. Divergence of developmental trajectories is triggered interactively by early social and ecological experience in a cooperative breeder. Proc. Natl. Acad. Sci. USA 114, E9300–E9307. https://doi.org/10.1073/pnas.1705934114.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. Eur. J. Pharmacol. 281, 81–88. https://doi.org/10.1016/0014-2999(95)00234-C.

Frazer, A., Hensler, J.G., 1999. Serotonin receptors. In: Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M.D. (Eds.), Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Lippincott-Raven, Philadelphia.

Garcia-Garcia, A.L., Newman-Tancredi, A., Leonardo, E.D., 2014. P5-HT1A receptors in mood and anxiety: recent insights into autoreceptor versus heteroreceptor function. Psychopharmacology 231, 623–636. https://doi.org/10.1007/s00213-013-3389-x.

Gerson, S.C., Baldessarini, R.J., 1980. Motor effects of serotonin in the central nervous system. Life Sci. 27, 1435–1451.

Groenewoud, F., Frommen, J.G., Josi, D., Tanaka, H., Jungwirth, A., Taborsky, M., 2016. Predation risk drives social complexity in cooperative breeders. Proc. Natl. Acad. Sci. USA 113, 4104–4109.

Hannon, J., Hoyer, D., 2008. Molecular biology of 5-HT receptors. Behav. Brain Res. 195, 198–213. https://doi.org/10.1016/j.bbr.2008.03.020.

Hellstrom, I.C., Dhir, S.K., Diorio, J.C., Meaney, M.J., 2012. Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. Philos. Trans. R. Soc. B Biol. Sci. 367, 2495–2510. https://doi.org/10.1098/rstb.2012.0223.

Helsley, S., Siegel, T.L., Fiorella, D., Rabin, R.A., Winter, J.C., 1998. WAY-100635 reverses 8-OH-DPAT-induced performance impairment in the radial maze. Progr. Neuro-Psychopharmacol. Biol. Psychiat. 22, 1179–1184. https://doi.org/10.1016/S0278-5846(98)00068-2.

Hjorth, S., Magnusson, T., 1988. The 5-HT1A receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. Naunyn Schmiedeberg's Arch. Pharmacol. 338, 463–471. https://doi.org/10.1007/BF00179315.

Hofmann, H.A., Beery, A.K., Blumstein, D.T., Couzin, I.D., Earley, R.L., Hayes, L.D., Hurd, P.L., Lacey, E.A., Phelps, S.M., Solomon, N.G., Taborsky, M., Young, L.J., Rubenstein, D.R., 2014. An evolutionary framework for studying mechanisms of social behavior. Trends Ecol. Evol. 29, 581–589. https://doi.org/10.1016/j.tree.2014.07.008.

Kasper, C., Kölliker, M., Postma, E., Taborsky, B., 2017. Consistent cooperation in a cichlid fish is caused by maternal and developmental effects rather than heritable genetic variation. Proc. R. Soc. B Biol. Sci. 284, 20170369. https://doi.org/10.1098/rspb.2017.0369.

Kasper, C., Colombo, M., Aubin-Horth, N., Taborsky, B., 2018a. Brain activation patterns following a cooperation opportunity in a highly social cichlid fish. Physiol. Behav. 195, 37–47. https://doi.org/10.1016/j.physbeh.2018.07.025.

Kasper, C., Hebert, F.O., Aubin-Horth, N., Taborsky, B., 2018b. Divergent brain gene expression profiles between alternative behavioural helper types in a cooperative breeder. Mol. Ecol. 27, 4136–4151. https://doi.org/10.1111/mec.14837.

Kelly, A.M., Vitousek, M.N., 2017. Dynamic modulation of sociality and aggression: an examination of plasticity within endocrine and neuroendocrine systems. Philos. Trans. R. Soc. B Biol. Sci. 372 https://doi.org/10.1098/rstb.2016.0243.

Kiser, D., Steemers, B., Branchi, I., Homberg, J.R., 2012. The reciprocal interaction between serotonin and social behaviour. Neurosci. Biobehav. Rev. 36, 786–798. https://doi.org/10.1016/j.neubiorev.2011.12.009.

Kravitz, E.A., 2000. Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. J. Comp. Physiol. A. 186, 221–238. https://doi.org/10.1007/s003590050423.

Lanfumey, L., Hamon, M., 2000. Central 5-HT1A receptors: regional distribution and functional characteristics. Nucl. Med. Biol. 27, 429–435. https://doi.org/10.1016/S0969-8051(00)00107-4.

Larke, R.H., Maninger, N., Ragen, B.J., Mendoza, S.P., Bales, K.L., 2016. Serotonin 1A agonism decreases affiliative behavior in pair-bonded titi monkeys. Horm. Behav. 86, 71–77. https://doi.org/10.1016/j.yhbeh.2016.10.001.

Larson, E.T., Summers, C.H., 2001. Serotonin reverses dominant social status. Behav. Brain Res. 121, 95–102. https://doi.org/10.1016/S0166-4328(00)00393-4.

Lillesaar, C., 2011. The serotonergic system in fish. J. Chem. Neuroanat. 41, 294–308. https://doi.org/10.1016/j.jchemneu.2011.05.009.

Lopez-Mendoza, D., Aguilar-Bravo, H., Swanson, H.H., 1998. Combined effects of gepirone and (+)WAY 100135 on territorial aggression in mice. Pharmacol. Biochem. Behav. 61, 1–8. https://doi.org/10.1016/S0091-3057(97)00563-7.

Lucki, I., 1992. 5-HT1 receptors and behavior. Neurosci. Biobehav. Rev. 16, 83–93. https://doi.org/10.1016/S0149-7634(05)80055-7.

Lucki, I., 1998. The spectrum of behaviors influenced by serotonin. Biol. Psychiat. 44, 151–162. https://doi.org/10.1016/S0006-3223(98)00139-5.

Medeiros, L.R., McDonald, M.D., 2013. Cortisol-mediated downregulation of the serotonin 1A receptor subtype in the Gulf toadfish *Opsanus beta*. Comp. Biochem. Physiol. A 164, 612–621. https://doi.org/10.1016/j.cbpa.2013.01.014.

Nichols, D.E., Nichols, C.D., 2008. Serotonin receptors. Chem. Rev. 108, 1614–1641. https://doi.org/10.1021/cr078224o.

Olivier, B., Mos, J., van Oorschot, R., Hen, R., 1995. Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 28, 80–90. https://doi.org/10.1055/s-2007-979624.

Paula, J.R., Messias, J.P., Grutter, A.S., Bshary, R., Soares, M.C., 2015. The role of serotonin in the modulation of cooperative behavior. Behav. Ecol. 26, 1005–1012. https://doi.org/10.1093/beheco/arv039.

Prasad, P., Ogawa, S., Parhar, I.S., 2015. Role of serotonin in fish reproduction. Front. Neurosci. 9, 195. https://doi.org/10.3389/fnins.2015.00195.

Puglisi-Allegra, S., Andolina, D., 2015. Serotonin and stress coping. Behav. Brain Res. 277, 58–67. https://doi.org/10.1016/j.bbr.2014.07.052.
R Core Team, 2017. R: A Language and Environment for Statistical Computing. https://www.R-project.org/.
Romano, E., Ruocco, L.A., Nativio, P., Lacivita, E., Ajmone-Cat, M.A., Boatto, G., Nieddu, M., Tino, A., Sadile, A.G., Minghetti, L., Passarelli, F., Leopoldo, M., Laviola, G., Adriani, W., 2014. Modulatory effects following subchronic stimulation of brain 5-HT7-R system in mice and rats. Rev. Neurosci. 25, 383–400. https://doi.org/10.1515/revneuro-2014-0007.
Sperry, T.S., Thompson, C.K., Wingfield, J.C., 2003. Effects of acute treatment with 8-OH-DPAT and fluoxetine on aggressive behaviour in male song sparrows (*Melospiza melodia morphna*). J. Neuroendocrinol. 15, 150–160. https://doi.org/10.1046/j.1365-2826.2003.00968.x.
Sporer, K.A., 1995. The serotonin syndrome. Drug Saf. 13, 94–104. https://doi.org/10.2165/00002018-199513020-00004.
Stewart, A.M., Cachat, J., Gaikwad, S., Robinson, K.S.L., Gebhardt, M., Kalueff, A.V., 2013. Perspectives on experimental models of serotonin syndrome in zebrafish. Neurochem. Int. 62, 893–902. https://doi.org/10.1016/j.neuint.2013.02.018.
Symonds, M.R.E., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behav. Ecol. Sociobiol. 65, 13–21. https://doi.org/10.1007/s00265-010-1037-6.
Taborsky, M., 2016. Cichlid fishes: a model for the integrative study of social behavior. In: Koenig, W.D., Dickinson, J.L. (Eds.), Cooperative Breeding in Vertebrates. Cambridge University Press, Cambridge, pp. 272–293. https://doi.org/10.1017/CBO9781107338357.017.
Taborsky, B., Arnold, C., Junker, J., Tschopp, A., 2012. The early social environment affects social competence in a cooperative breeder. Anim. Behav. 83, 1067–1074. https://doi.org/10.1016/j.anbehav.2012.01.037.
Teles, M.C., Dahlbom, S.J., Winberg, S., Oliveira, R.F., 2013. Social modulation of brain monoamine levels in zebrafish. Behav. Brain Res. 253, 17–24. https://doi.org/10.1016/j.bbr.2013.07.012.
Tricklebank, M.D., Forler, C., Fozard, J.R., 1984. The involvement of subtypes of the 5-HT1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-Propylamino) tetralin in the rat. Eur. J. Pharmacol. 106, 271–282. https://doi.org/10.1016/0014-2999(84)90714-3.
Winberg, S., Lepage, O., 1998. Elevation of brain 5-HT activity, POMC expression, and plasma cortisol in socially subordinate rainbow trout. Am. J. Physiol. 274, R645–R654. https://doi.org/10.1152/ajpregu.1998.274.3.R645.
Winberg, S., Thörnqvist, P.-O., 2016. Role of brain serotonin in modulating fish behavior. Curr. Zool. 62, 317–323. https://doi.org/10.1093/cz/zow037.
Zhang, Y., Gray, T.S., Souza, D.N.D., Carrasco, G.A., Damjanoska, K.J., Dudas, B., Garcia, F., Zainelli, G.M., Hanley, N.R.S., Battaglia, G., Muma, N.A., Van de Kar, L. D., 2004. Desensitization of 5-HT 1A receptors by 5-HT 2A receptors in neuroendocrine neurons in vivo. Pharmacology 310, 59–66. https://doi.org/10.1124/jpet.103.062224.5-HT.
Zhao, Y., Bijlsma, E.Y., ter Heegde, F., Verdouw, M.P., Garssen, J., Newman-Tancredi, A., Groenink, L., 2019. Activation of somatodendritic 5-HT1A autoreceptors reduces the acquisition and expression of cued fear in the rat fear-potentiated startle test. Psychopharmacology 236, 1171–1185. https://doi.org/10.1007/s00213-018-5124-0.
Zöttl, M., Heg, D., Chervet, N., Taborsky, M., 2013. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. Nat. Commun. 4, 1341. https://doi.org/10.1038/ncomms2344.
Zuideveld, K.P., Treijtel, N., Maas, H.J., Gubbens-Stibbe, J.M., Peletier, L.A., van Der Graaf, P.H., Danhof, M., 2002. A competitive interaction model predicts the effect of WAY- propylamino tetralin-induced hypothermia. J. Pharmacol. Exp. Ther. 300, 330–338. https://doi.org/10.1124/jpet.300.1.330.
Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R (Springer Science & Business Media).