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Cognitive appraisal in fish: stressor predictability modulates the physiological and neurobehavioural stress response in sea bass

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The role of cognitive factors in triggering the stress response is well established in humans and mammals (aka cognitive appraisal theory) but very seldom studied in other vertebrate taxa. Predictability is a key factor of the cognitive evaluation of stimuli. In this study, we tested the effects of stressor predictability on behavioral, physiological and neuromolecular responses in the European sea bass (*Dicentrarchus labrax*). Groups of four fish were exposed to a predictable (signalled) or unpredictable (unsignalled) stressor. Stressor predictability elicited a lower behavioural response and reduced cortisol levels. Using the expression of immediate early genes (*c-fos*, *egr-1*, *bdnf* and *npas4*) as markers of neuronal activity, we monitored the activity of three sea bass brain regions known to be implicated in stressor appraisal: the dorsomedian telencephalon, Dm (putative homologue of the pallial amygdala); and the dorsal (Dld) and ventral (Dlv) subareas of the dorsolateral telencephalon (putative homologue of the hippocampus). The activity of both the Dm and Dlv significantly responded to stressor predictability, suggesting an evolutionarily conserved role of these two brain regions in information processing related to stressor appraisal. These results indicate that stressor predictability plays a key role in the activation of the stress response in a teleost fish, hence highlighting the role of cognitive processes in fish stress.

1. Introduction

The literature on stress biology has long established the role of cognitive factors on triggering the stress response, defined as a response of the organism to regain homeostasis when exposed to a homeostasis threatening stimulus or event (aka stressor) [1]. Since the 1970s, it became clear that the cognitive appraisal of stimuli is a key mechanism in the activation of the stress response [2,3]. According to this perspective, it is not the intrinsic physical characteristics of the stimulus that trigger a response but rather the evaluation of what that stimulus or event means to that organism at that moment in time, which depends on stored information in memory about relations between stimuli (i.e. stimulus–stimulus learning or classical conditioning) and about relations between responses and stimuli (i.e. stimulus–response learning or instrumental conditioning) [4]. Therefore, the same stimulus may elicit or not a stress response depending on how it is appraised by the individual. An ‘alarm’ response would occur when expectancies, based on perceived contingencies

between stimuli (i.e. stimulus expectancies) and between stimulus and response (i.e. response expectancies), are not met (i.e. when there is a discrepancy between expected situation and perceived situation). Hence, stimulus predictability, which refers to high levels of perceived probability of occurrence of the expected event, and stimulus controllability, which refers to high levels of perceived probability for response outcomes, play a major role on the appraisal of stimuli as aversive or not [5]. Interestingly, the role of cognitive variables in the activation of a stress response was first investigated in laboratory animals, in particular in rodents (e.g. [6]), and then extended to humans (e.g. [7]).

In recent decades, the role of cognitive variables in the activation of stress responses as well as in triggering responses to appetitive events has been framed under a theory of cognitive appraisal. According to this theory, individuals continuously monitor the environment using a set of stimulus evaluation checks (e.g. intrinsic valence, novelty, prediction error and capacity for control) in order to evaluate the valence (positive/ negative) and salience (high/ low) of detected stimuli (primary appraisal), and also assess the available organismal resources to deal with them (secondary appraisal) [8–10]. While the appraisal concept has already been applied to the study of stress and emotional behaviour in animals, mainly in mammals (see [8] for a recent review), in fish, the whole concept of psychological stress has been rarely addressed [5,11–16]. However, empirical evidence for the occurrence of each of the stimulus evaluation checks involved in primary appraisal has been described in fish. The appraisal of the intrinsic valence of stimuli can be demonstrated by learned approach/avoidance behaviours, and these have been described in different fish species [17,18]. The use of the three cues that signal stimulus novelty have also been documented in fish: the effects of predictability in modulating the behavioural and physiological response to both aversive and appetitive stimuli have been described in the Mozambique tilapia (*Oreochromis mossambicus*) [12]; familiarity with conspecifics has been shown to modulate both exploratory behaviour and the response to a territorial intrusion, also in tilapia [19,20]; and the effect of controllability can be illustrated by rainbow trout (*Oncorhynchus mykiss*) that have the chance to actively avoid being defeated by a larger conspecific in a conditioning paradigm exhibiting a lower cortisol response to the conditioned stimulus, than those that cannot escape social defeat [21]. Finally, prediction error has recently been documented both in rainbow trout and in Atlantic salmon (*Salmo salar*) using a reward omission paradigm [15,16,22]. However, this evidence has so far not been explicitly presented as supporting the occurrence of cognitive appraisal in fish and the proximate (i.e. neural/physiological) bases of these cognitive appraisal processes have not been investigated yet in fish. Given the expected universality of stimulus evaluation checks across animals, it is now timely to characterize their occurrence across species and to implement comparative studies on the underlying neural mechanisms. Teleost fish offer an excellent opportunity for such comparative approach, given the divergent evolutionary path between ray-finned fish and tetrapods [23], and the homologies that have already been established between teleost and mammalian brain regions, that include some of the areas known to be involved in cognitive appraisal in mammals (i.e. amygdala and hippocampus [24–26]). Thus, the study of cognitive appraisal in fish will allow testing if the

same cognitive appraisal processes are present in evolutionarily divergent vertebrate taxa and if they share homologue neural mechanisms.

In this study, we tested the effect of predictability of a stressor on the behavioural and physiological stress response of European sea bass (*Dicentrarchus labrax*). Sea bass was used as a model in this study given its wide use in European aquaculture, which makes the results presented here not only of importance for the basic biology of fish stress but also to have translational value for the improvement of welfare of farmed fish. We have also characterized the pattern of neuronal activation (using the expression of immediate early genes (IEGs) as markers of neuronal activation) of two brain regions that are homologous to mammalian brain regions known to be involved in cognitive appraisal in mammals, namely the dorsomedial telencephalon (Dm, putative teleost homologue of the mammalian amygdala) and the dorsolateral telencephalon (Dl, putative teleost homologue of the mammalian hippocampus), in order to test if brain regions involved in cognitive appraisal are evolutionarily conserved. Given that predictability is a key stimulus evaluation check in cognitive appraisal theory, its occurrence in fish will also be proof for the occurrence of cognitive appraisal in fish.

2. Material and methods

(a) Experimental fish and maintenance

A batch of sea bass with an initial body weight of 0.5 ± 0.3 g (mean \pm s.d.) hatched at the experimental research station of IFREMER in Palavas-les-Flots (France) were transported to Ramalhete Research Station (CCMAR, Faro, Portugal). Fish were reared in 500 l tanks in an open water circuit with constant aeration through air stones (temperature of $21 \pm 5^\circ\text{C}$, salinity of $35 \pm 1\text{‰}$, dissolved oxygen above 75% and a 12 L:12D photoperiod) during ten months before the experiments. Fish were initially fed at 10% of body weight with commercial diets (Aquadgold, Aquasoja, Sorgal SA, Portugal), and later food amount was readjusted until 3% of body weight in accordance with their growth. A total of 72 fish with a body weight of 44.58 ± 6.36 g (mean \pm s.d.) at the start of the experiments were used.

(b) Experimental design and conditioning procedures

The effects of predictability on the fish stress response were tested in groups of four individuals randomly chosen from the reared tank. The experiment occurred between May and June of 2013 ($T(^{\circ}\text{C}) = 21.89 \pm 1.77$, $\text{DO}(\%) = 86 \pm 6$ and $\text{pH} = 8.13 \pm 0.15$). Eighteen experimental glass aquaria ($70 \times 40 \times 30$ cm) were used under the same housing conditions as described above, except for the fact that no airflow was supplied since the water flow rate of 2.5 l min^{-1} was sufficient to guarantee oxygen saturation. A net, with the same dimension as the lateral wall, was settled in one side of each aquarium at the beginning of the experiment to be used as a confinement net. All aquaria walls were covered with opaque plastic to avert visual contact between the animals and the experimenters. The fish were fed at $3\% \text{ Bw}^{-1}$ daily, divided by two meals at 08.00 h and 18.00 h. Water quality was analysed for nitrites (less than 0.1 mg l^{-1}) and ammonia (less than 0.1 mg l^{-1}) every three days. Temperature, oxygen saturation and pH were daily checked before the cleaning routines performed 1 h after the second meal.

One month before the experiments, 72 fish were tagged under anaesthesia with a 1 cm floy tag (Floy Tag Manufacturing, Seattle, USA) and with a multicolour pearl attached behind the

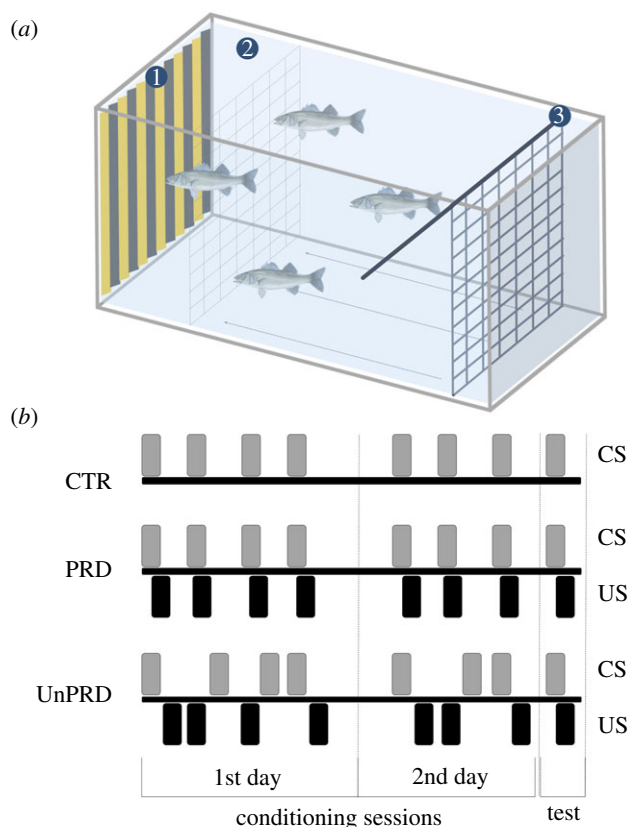


Figure 1. Overview of the protocol and experimental conditions used to test predictability as an appraisal modulator of aversive events in sea bass, *Dicentrarchus labrax*. (a) Schematic of the experimental tank. 1. Visual cue CS, settled at the time of conditioning. 2. Confinement area, correspondingly to 15% of the aquarium volume. 3. Confinement net US, settled at the beginning of the trial. (b) Description of the procedures used to test predictability: CTR, control; PRD, predictability; UnPRD, unpredictability. (Online version in colour.)

dorsal fin. Three experimental conditions were tested: control (CTR), predictability (PRD) and unpredictability (UnPRD). The experiment lasted 14 days, in which six groups of 4 fish each were used in each experimental condition (control conditions, CTR; predictable conditions, PRD; unpredictable conditions, UnPRD; $n=24$ fish per treatment). After the acclimation period of 12 days, the experimental period occurred in the following 2 days and involved four training sessions in the first day (at 10.00 h, 12.00 h, 14.00 h and 16.00 h), and three training sessions (at 10.00 h, 12.00 h and 14.00 h) and one test session (at 16.00 h) on the second day (see figure 1 for an overview of experimental procedures). To create the predictable and unpredictable treatments, two different training procedures were used. In the predictable treatment, a delay conditioning protocol was used for fish to learn to associate a visual cue (CS), which consisted of a yellow and black striped card with the same size as the lateral wall of the aquaria (40×30 cm), with a stressor (US: confinement promoted by a rigid net of the same size). The net was settled on the opposite side from the CS, and the US obtained by moving the net towards the CS until the fish were restrained in 15% of the aquarium volume. During the procedure, the CS remained in view for 1 min before the occurrence of US, and overlapped 1 min with it (see figure 1 for the schematic of the experimental tank and conditioning procedures). In the unpredictable treatment, fish were presented with the same visual sign but temporally dissociated from the stressor (i.e. 30 min before or after in a random way). In the control groups, fish were only subjected to the CS without US presentation. The number of conditioning trials used was

based on preliminary tests that indicated that 5–6 trials were enough for aversive conditioning in this species. In the test session, fish from both experimental treatments (PRD and UnPRD) were exposed to the visual cue together with the stressor, and fish from control were subjected only to the CS to discard the effect of the CS on fish responses during the test session.

(c) Behavioural observations

Fish behaviour was video recorded right before the first training session and during the test session using video cameras (TVCCD-623-COL, Monacor, Denmark) and webcams (HD C310 Logitech) positioned 1 m above the tank. Videos were subsequently analysed using multi-event recorder software (Observer XT® from Noldus, Netherlands). The response to the visual cue was assessed using the following behavioural measurements: (1) time spent in freezing behaviour (i.e. time fish spent immobile, with or without fin movements, either on the bottom or in the water column); (2) escape behaviour, that is increase of fish swimming speed and movements towards the bottom of the tanks or towards the tank walls, or moving the body against the tank walls; (3) shoal cohesion, quantified through a proximity metric, defined as the distance variation between individuals within the shoal structure, and measured in an arbitrary scale (1 = low, mean distance greater than 15 cm apart; 2 = medium, $5 \text{ cm} < \text{mean distance} < 15 \text{ cm}$ apart; 3 = high, mean distance $< 5 \text{ cm}$ apart); and (4) exploratory behaviour, measured according to Galhardo *et al.* [12], following the formula:

$$\frac{A}{t_{\text{maximum}}}$$

where A is the arithmetic mean of the time fish spent in each one of three previously delimited areas of the tank (confinement net area; centre of the tank; and visual cue area), and t_{maximum} is the maximum time found for any of the areas tested. When this ratio is close to 1 it indicates high exploratory behaviour, and when it is close to 0 it indicates low exploratory behaviour.

(d) Blood sampling and plasma cortisol analysis

For each treatment, 30 min after the test session, fish were rapidly caught at the same time through a soft net with the same width as the experimental tank to reduce net chasing bias; they were euthanized with an overdose of 2-phenoxyethanol (1%, Sigma-Aldrich) and blood was immediately collected from the caudal vein and centrifuged at RT for 25 min at 2000g. Plasma was stored at -80°C until further processing. Plasma cortisol levels were measured using a commercial ELISA kit (RE52061, IBL Hamburg, Germany), with a sensitivity of 2.5 ng ml^{-1} and intra- and inter-assay coefficients of variation (CV) were 2.9% and 3.5%, respectively.

(e) Brain microdissection and gene expression analysis

Eight individuals from each experimental treatment were randomly selected for the assessment of IEGs mRNA expression in brain regions of interest (see below). Fish were sacrificed and the skull with the brain inside was immediately imbedded in Tissue-Tek and kept at -80°C until further processing. Brain telencephalon was sliced through $150 \mu\text{m}$ thick cryostat (Leica, CM 3050S) coronal sections, from which the medial part of the dorsal telencephalon (Dm), the dorsal division of the lateral telencephalon (Dld) and the ventral division of the lateral telencephalon (Dlv) (see electronic supplementary material, figure S1) were microdissected with modified 25 G steel needles using a micropunching technique previously established in the

laboratory [27]. These regions of interest in the brain were identified and classified following the available brain atlas for sea bass [28]. Total tissue was collected directly into lysis buffer from Qiagen Lipid Tissue Mini Kit (no. 74804; Valencia, CA) and total RNA extracted from the samples, with some adjustments to the manufacturer's instructions (see electronic supplementary material for detailed procedures). RNA from each sample was then reverse transcribed to cDNA (BioRad iScript cDNA Synthesis Kit; Valencia, CA) accordingly to manufacturer's instructions and used as a template for quantitative polymerase chain reactions (qPCR) of *egr-1*, *c-fos*, *bdnf* and *npas4*, using the geometric mean of the expression of two previously established housekeeping genes, *ef1a* and *18S* (see electronic supplementary material, table S1 for primer sequences and for qPCR conditions). The abundance of the internal control genes was stable across experimental treatments. All reactions were run in duplicate and controls without DNA templates were run to verify the absence of cDNA contamination. Fluorescence cycle thresholds (CTs) were automatically measured and relative expression of the target genes were calculated using the $2^{-\Delta Ct}$ method [29]. Primers efficiency was calculated for each qRT-PCR reaction using Light Cycler 480 II inner software.

(f) Statistical analysis

Parametric assumptions of normality and homoscedasticity of the data were confirmed by analysis of the residuals. Homogeneity of variances was checked by Levene's test. Log, $\log(X+1)$ or arcsine transformations were used to match parametric assumptions when required (time in freezing (arc-sin transformed), escape behaviour and exploratory behaviour ($\log(X+1)$ transformed), Shoal cohesion, plasma cortisol concentration and IEGs mRNA expression (log-transformed)). LMM analyses were used to assess the effect of predictability (i.e. PRD versus CTR; PRD versus UnPRD; CTR versus UnPRD) on the behavioural variables before any stimulation. The same analysis was performed for a test session on the behavioural variables, on cortisol levels and on IEGs mRNA expression (*egr-1*, *c-fos*, *bdnf* and *npas4*) in each brain region (Dm, Dld and Dlv). Given that we have used more than one individual from the same experimental tank in each treatment, pseudo replication concerns could be raised. We accounted for sampling dependence by adding a random effect for the 'tank' factor in each LMM. In general, we did not find an effect of the 'tank' variable on the measured responses. All LMM were estimated using the restricted maximum-likelihood method. *A priori* planned comparisons with *p*-values adjusted following the Benjamini and Hochberg's method were used to test for specific differences between experimental conditions, namely: PRD versus CTR; PRD versus UnPRD; CTR versus UnPRD. Pearson test was used to assess correlations among variables. Descriptive statistics are expressed as mean \pm s.e.m. The LMM and planned comparisons were performed using R (R Development Core Team) and GraphPad Prism v. 6.0 for windows was used for chart building and figures layout.

3. Results

(a) Effects of stressor predictability on fish behaviour

Analyses of fish behaviour during the 2 min preceding the first training session (i.e. before any stimulation or manipulation of the fish) showed no significant differences between the experimental treatments PRD, UnPRD and CTR (time freezing: $F_{(2,54)}=0.36$, $p=0.69$; escape events: $F_{(2,54)}=0.44$, $p=0.64$; exploratory behaviour: $F_{(2,54)}=0.31$, $p=0.73$). In the same way, shoal cohesion before training also

did not show differences between experimental conditions ($F_{(2,54)}=0.01$, $p=0.98$).

The behaviour displayed by fish during exposure to the visual cue in the test session was markedly different between experimental treatments (figure 2; electronic supplementary material, table S2). Fish in the predictable treatment spent less time in freezing, and showed less escape attempts and more exploratory behaviour than fish in the unpredictable treatment (figure 2*a-c*; electronic supplementary material, table S2). Moreover, in this experiment, time in freezing and escape attempts were positively correlated ($R_p=0.721$, $n=72$, $p<0.001$) and exploratory behaviour and time in freezing were negatively correlated ($R_p=-0.299$, $n=48$, $p=0.011$). Finally, shoal cohesion was higher in the unpredictable treatment over predictable and control conditions (figure 2*d*; electronic supplementary material, table S2). Moreover, there was a negative correlation between shoal cohesion and exploratory behaviour ($R_p=-0.427$, $n=72$, $p<0.001$).

(b) Effects of stressor predictability on fish physiology

Fish exposed to unpredictable stressors had higher cortisol levels than fish exposed to predictable stressors and to the levels found for the control group (electronic supplementary material, table S2; figure 3). Cortisol was positively correlated with time in freezing, shoal cohesion and frequency of escape events ($R_p=0.355$, $n=68$, $p=0.003$; $R_p=0.371$, $n=68$, $p=0.002$ and $R_p=0.327$, $n=68$, $p=0.006$, respectively), whereas a negative correlation was found with exploratory behaviour ($R_p=-0.656$, $n=68$, $p=0.001$).

(c) Effects of stressor predictability on brain activation

Stressor predictability induced significant changes in the expression levels of IEGs, with an upregulation of *egr-1* at Dm associated with stressor unpredictability and a decrease of *npas4* at Dlv associated with stressor predictability (figure 4). Moreover, both predictable and unpredictable stress-induced an upregulation of *egr-1* in Dld and a downregulation of *c-fos* in Dlv (figure 4). *Bdnf* expression did not respond to any of the stress treatments (electronic supplementary material, table S2).

(d) Correlations between predictability-driven behavioural, physiological and brain activation measures

A positive correlation was found between time in freezing and escape behaviour ($R_p=0.853$, $n=24$, $p<0.001$), and a negative correlation was found between exploratory behaviour and both shoal cohesion ($R_p=-0.593$, $n=24$, $p=0.002$) and cortisol ($R_p=-0.581$, $n=24$, $p=0.003$). Regarding neuronal plasticity, a negative correlation between cortisol and both *c-fos* and *bdnf* in the Dlv was also found ($R_p=-0.487$, $n=20$, $p=0.029$; $R_p=-0.473$, $n=22$, $p=0.026$, respectively).

4. Discussion

In this study, we have shown that stressor predictability modulates the stress response measured at the behavioural, physiological and neural levels. Fish exposed to the unpredictable stressor showed higher freezing and more escape behaviours, higher shoal cohesion, less exploratory

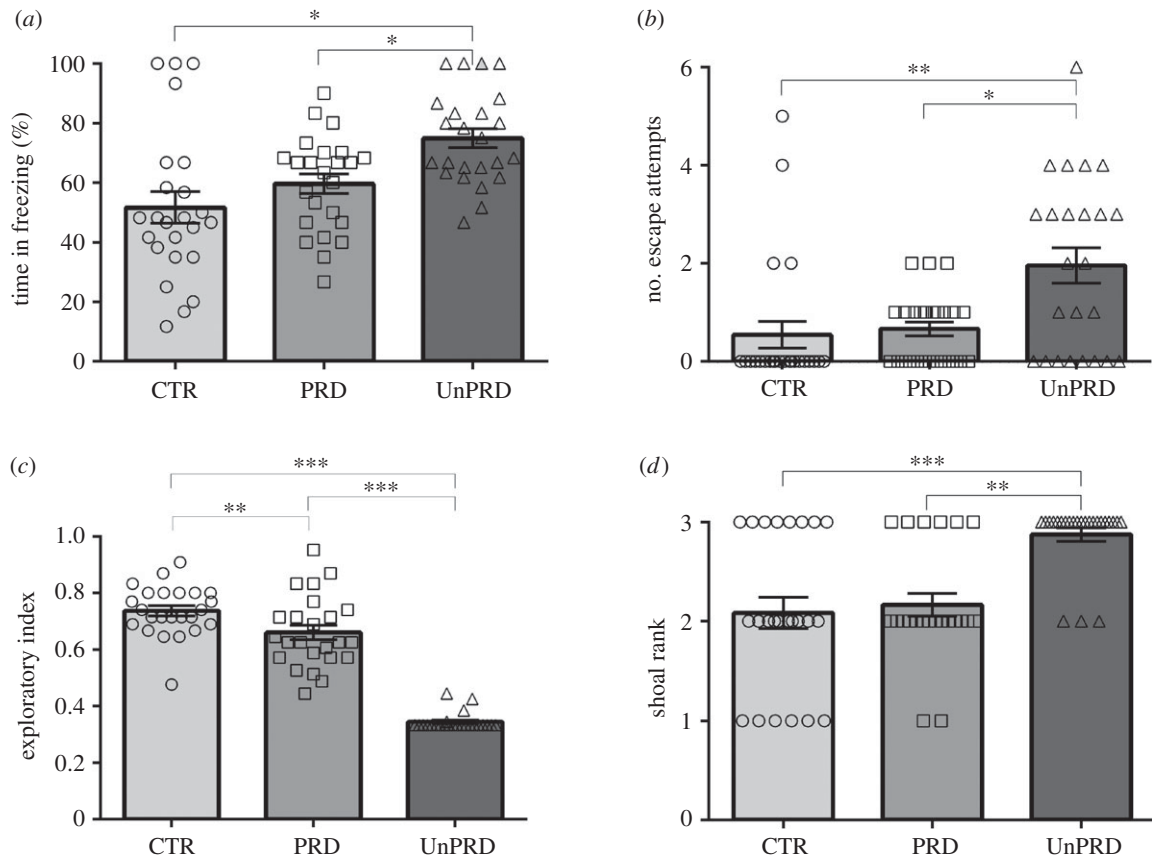


Figure 2. Behavioural responses (mean \pm s.e.m.) of fish in social groups towards predictable and unpredictable aversive stimuli (CTR, control; PRD, predictable; UnPRD, unpredictable): (a) time in freezing; (b) escape attempts; (c) exploratory behaviour (measured by the ratio between the arithmetic mean of the time spent in each area of the experimental tank, by the higher time spent measured of such areas); and (d) Shoal cohesion rank for fish tested under social conditions (1—low cohesion; 2—medium cohesion; 3—high cohesion). Significant differences between treatments are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). All descriptive statistics are mean \pm s.e.m.

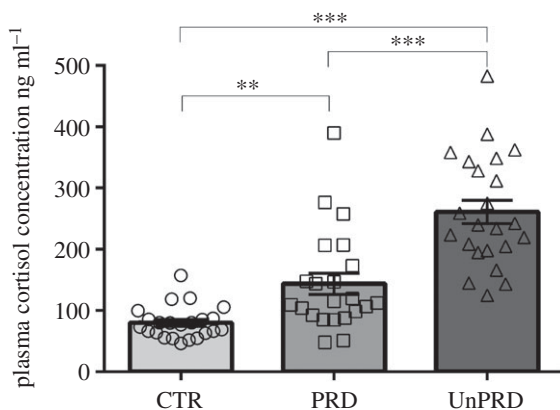


Figure 3. Plasma cortisol responses (mean \pm s.e.m.) of fish in social groups towards predictable and unpredictable aversive stimuli (CTR, control; PRD, predictable; UnPRD, unpredictable). Significant differences between treatments are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

behaviour, higher physiological reactivity and more activation of the Dm as indicated by the expression of *egr1*. Therefore, an unpredictable stressor seems to trigger a higher stress response both in terms of the activation of the hypothalamic–pituitary–interrenal axis (HPI) and of the activation of a brain region putatively involved in the appraisal of the stressor, such as the Dm (fish homologue of the tetrapod pallial amygdala). Thus, predictability seems to reduce the behavioural response to stress.

The effects of stressor predictability have been extensively studied both in humans and in animals, and consistently the results have shown that prediction reduces the stress effects of aversive experiences [30,31]. For example, in the rat, which was the original model in which stressor predictability has been studied, it reduces the behavioural responses to stress, as well as detrimental consequences of stress such as pain reactivity, immunosuppression, gastric ulceration and colonic motility (e.g. [6,32–36]). Similar results have been found subsequently in other mammalian species (e.g. sheep [37]; dogs [38]; horses [39]; pigs [40]). However, fewer studies have addressed such effects in non-mammalian vertebrates (e.g. birds [41]), and among fish, the few studies available have produced contrasting results. While in this study, in conformity with previous studies in Mozambique tilapia, Gilthead sea bream or in rainbow trout (e.g. [5,12,16]), stressor predictability buffers the stress response, in Atlantic salmon no effect has been found (e.g. [13]). Given the fact that the two contrasting results occur within the same family (Salmonidae), these differences do not seem to reflect a phylogenetic difference but rather a species-specific effect. Interestingly, classic studies in this field have shown that when rats are given a choice between a signalled and an unsignalled foot shock they prefer the former [42,43], despite the fact that signalled shocks are perceived as more intense than unsignalled ones [44]. Thus, it looks like during primary appraisal different stimulus evaluation checks are not equally weighted, as in this case, appraisal of stimulus predictability seems to have overridden the perception of stimulus

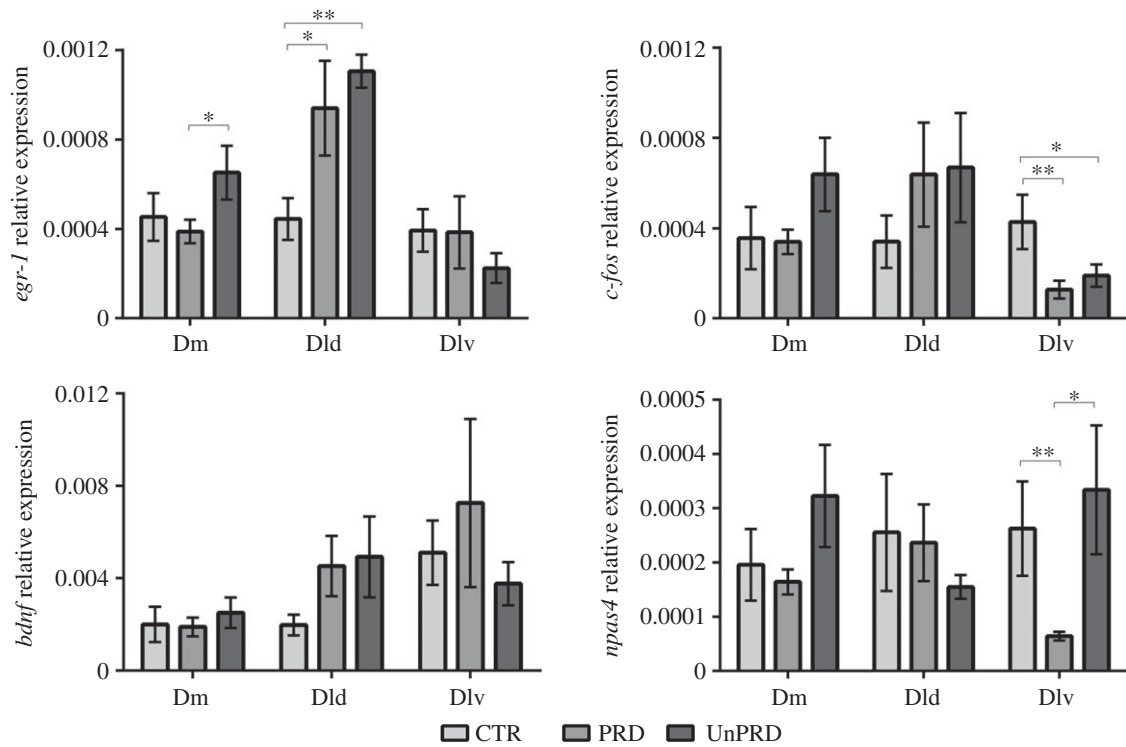


Figure 4. Expression (mean \pm s.e.m.) of the immediate early genes *egr-1*, *c-fos*, *bdnf* and *npas4* in different brain nuclei (Dm, medial part of the dorsal telencephalon; Dld, dorsal division of the lateral telencephalon; Dlv, ventral division of the lateral telencephalon) of fish in social groups towards predictable and unpredictable aversive stimuli (CTR, control; PRD, predictable; UnPRD, unpredictable). Significant differences in expression levels between experimental conditions (i.e. PRD versus UnPRD; PRD versus CTR; UnPRD versus CTR) are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

intensity. It is, therefore, important to extend the study of stressor predictability, and of cognitive appraisal in general, to other vertebrate species in order to assess how evolutionarily conserved these stimulus evaluation mechanisms are.

The amygdala together with the prefrontal cortex and the mesoaccumbens dopamine system have been implicated in the cognitive modulation of the stress response in mammals [45–48]. Given the lack of a neocortex and the absence of mid-brain dopaminergic neurons (Dahlström-Füxe's A10 nucleus, homologous to the mammalian mesolimbic ventral tegmental area) in fish (e.g. [49–51]), in this study, we have focused on the putative fish homologues of the mammalian amygdala (Dm) and hippocampus (DI).

In mammals, the amygdala plays a central role in emotional processes since it receives multi-modal sensory information, as well as inputs from the frontal cortex and the hippocampus, hence allowing it to assess the valence/salience of environmental stimuli in relation to expectations and to information in memory; and projects to the hypothalamus, striatum, hippocampus and cortex, thus coordinating physiological, cognitive and behavioural responses [52,53]. Similarly, in fish, the Dm also receives multimodal sensory inputs (e.g. olfactory, mechanosensory, auditory, electrosensory [54–58]), and has reciprocal connections with the hypothalamus [56,59–61]. Moreover, experimental lesions of Dm also impair emotional learning in fish, thus suggesting also a functional similarity between the teleost Dm and the mammalian amygdala [62]. Hence, the teleost Dm has been considered a putative homologue of the mammalian pallial amygdala [26,63]. The higher activation of Dm found in this study suggests a conserved role of this area in the cognitive appraisal of stressors. Our results further support the role

of the Dm in emotional processes in fish, in particular, the processing of aversive stimulus salience.

In mammals, the role of the hippocampus has been linked to the storage of repeated experiences, in particular, spatial memory [64]. In teleost fish, DI has been established as a homologue of the mammalian hippocampus, with experimental lesions in this area leading deficits in spatial learning, but not emotional or cue learning [62,65]. However, more precise analysis of the available evidence, based on extensive connections with septal nuclei and the preoptic area, the distribution patterns of histochemical and molecular markers and the patterns of neurogenesis and interneuron migration, suggests that this homology should be restricted to its ventral subdivision (Dlv) [66–68]. On the other hand, the dorsal subdivision of DI (Dld) seems to be specialized in the processing of visual information via a tectal loop and in the multimodal integration of visual information with other sensory modalities, given its afferents to other sensory organs [66,68]. Our results suggest that Dlv is also involved in stimulus appraisal, possibly due to its role in (reduced) memory storage of the predictable stimulus. Interestingly, the expression of *npas4*, an IEG involved in contextual learning [69], is significantly decreased in the predictable stressor treatment, suggesting a role for contextual learning of predictable stressors. Recently, it has been shown that *npas4* plays a critical role in experience-dependent regulation of structural and functional plasticity at mossy fibres-CA3 synapses in the mammalian hippocampus, during contextual memory formation [70].

It is interesting to note that both stressors (i.e. either predictable or unpredictable) elicited in parallel an increase in the expression of *egr-1* in the Dld and a decrease in expression of *c-fos* in Dlv. Although both *c-fos* and *egr-1* are transiently

expressed in response to neuronal activity, hence being widely used in the field as markers of neuronal activity, *c-fos* has usually a more ubiquitous expression with *egr-1* being regionally more restricted (e.g. [71]), and each of these genes plays different roles in neural plasticity. *C-fos* is involved in the regulation of transcription, and may mediate long-term effects of growth factors and membrane-depolarizing signals on neural activity [72]. *Egr-1* belongs to a family of transcriptional regulators (i.e. *egr-1*, *egr-2* and *egr-3*) involved in memory and learning [73]. Evidence from mutant mice suggests that *egr-1* is specifically required for long-term memory consolidation (e.g. [74–76]). The increase of activity in Dld (as reported by the upregulation of *egr-1*) in response to both stressor treatments can be associated with sustained higher arousal when repeatedly exposed to stressors, given the role of this area in the processing and integration of sensory stimuli. The decrease in activity in the Dlv (as reported by the downregulation of *c-fos*) suggests a reduced hippocampal-like memory storage during stress exposure, which is apparently further reduced in the predictable stressor treatment, as indicated by the reduction in *npas4* expression in the Dlv in the predictable stressor treatment discussed above.

Given the established role of *bdnf* in stress-induced neural plasticity [77], the lack of effects of stressor exposure on *bdnf* expression may be seen as surprising. However, in rodents, there are conflicting results regarding the effect of acute restraint as a stressor on *bdnf* expression, with some studies reporting increases and others decreases in expression, with variation also regarding brain regions [77]. Moreover, in rodents, the increase in *bdnf* expression has been detected in the hippocampus 1 h after stressor exposure [78]. Thus, our sampling time point may have failed to capture a putative *bdnf* response to our stressor treatments.

Finally, it should be mentioned that the loss of predictability (predictable followed by unpredictable conditions) has also been reported to act as a stressor by itself, being even more detrimental than unpredictable regimes [79,80]. In fish, a recent work has demonstrated that Atlantic salmon,

increase aggressive behaviour after reward omission [15]. Thus, predictability not only of aversive but also of appetitive stimuli (e.g. feeding regimes) seem to play a major role in stress management and should be considered in the handling of farmed fish as a way to stress reduction (see [79] for a review on the impact of predictability of animal welfare). In line with this, controllability, another key component of stimuli appraisal, is recognized to increase coping ability by combining the individual's affective state and the environmental conditions for the appraisal process [81,82]. In fish, it was demonstrated that an aversive event is less stressful when the animal exerts control over it, likely reducing negative emotional responses and permitting adjusting their coping responses to the environmental conditions [83].

In summary, in this study, we have shown that stressor predictability modulates the stress response at multiple levels (behavioural, physiological and neuronal) in sea bass, which supports the occurrence of cognitive appraisal of environmental stimuli in fish and highlights the need to consider psychological stress in the handling of farmed fish.

Ethics. All applicable international, national and/or institutional guidelines, with the permit no. 0420/00/000-n.9909/11/2009, for the care and use of animals were followed.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors' contributions. R.O., S.M. and M.C. designed research; M.C. and S.M. performed research; M.C. and A.F. carried out the molecular laboratory work; M.C., T.S. and G.A.O. carried out the statistical analyses; C.C.V.O., S.R. and S.M. critically revised the manuscript; R.O. and M.C. wrote the paper.

Competing interests. We declare we have no competing interests.

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References

- Koolhaas JM *et al.* 2011 Stress revisited: a critical evaluation of the stress concept. *Neurosci. Biobehav. Rev.* **35**, 1291–1301. (doi:10.1016/j.neubiorev.2011.02.003)
- Mason JW. 1968 A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosom. Med.* **30**(Suppl.), 576–607. (doi:10.1097/00006842-196809000-00020)
- Weiss JM. 1972 Influence of psychological variables on stress induced pathology. In *Physiology, emotion and psychosomatic illness* (eds P Porter, J Knight), pp. 253–264. Amsterdam, the Netherlands: CIBA Foundation Symposium.
- Ursin H, Eriksen HR. 2004 The cognitive activation theory of stress. *Psychoneuroendocrinology* **29**, 567–592. (doi:10.1016/S0306-4530(03)00091-X)
- Cerqueira M *et al.* 2017 Cognitive appraisal of environmental stimuli induces emotion-like states in fish. *Sci. Rep.* **7**, 13181. (doi:10.1038/s41598-017-13173-x)
- Weiss JM. 1970 Somatic effects of predictable and unpredictable shock. *Psychosom. Med.* **32**, 397–406. (doi:10.1097/00006842-197007000-00008)
- Lazarus RS. 1999 *Stress and emotion: a new synthesis*. New York, NY: Springer Publishing Company.
- Faustino AI, Oliveira GA, Oliveira RF. 2015 Linking appraisal to behavioral flexibility in animals: implications for stress research. *Front. Behav. Neurosci.* **9**, 104. (doi:10.3389/fnbeh.2015.00104)
- Mendl M, Burman OHP, Paul ES. 2010 An integrative and functional framework for the study of animal emotion and mood. *Proc. R. Soc. B* **277**, 2895–2904. (doi:10.1098/rspb.2010.0303)
- Moors A, Ellsworth PC, Scherer KR, Frijda NH. 2013 Appraisal theories of emotion: state of the art and future development. *Emotions Rev.* **5**, 119–124. (doi:10.1177/1754073912468165)
- Galhardo L, Oliveira RF. 2009 Psychological stress and welfare in fish. *Annu. Rev. Biomed. Sci.* **11**, 1–20. (doi:10.1146/annurev-bioeng-061008-124927)
- Galhardo L, Vital J, Oliveira RF. 2011 The role of predictability in the stress response of a cichlid fish. *Physiol. Behav.* **102**, 367–372. (doi:10.1016/j.physbeh.2010.11.035)
- Madaro A *et al.* 2016 Effect of predictability on the stress response to chasing in Atlantic salmon (*Salmo salar* L.) parr. *Physiol. Behav.* **153**, 1–6. (doi:10.1016/j.physbeh.2015.10.002)
- Madaro A *et al.* 2015 Stress in Atlantic salmon: response to unpredictable chronic stress. *J. Exp. Biol.* **218**, 2538–2550. (doi:10.1242/jeb.120535)
- Vindas MA *et al.* 2014 Frustrative reward omission increases aggressive behaviour of inferior fighters.

- Proc. Biol. Sci.* **281**, 20140300. (doi:10.1098/rspb.2014.0300)
16. Vindas MA *et al.* 2014 Coping with unpredictability: dopaminergic and neurotrophic responses to omission of expected reward in Atlantic Salmon (*Salmo salar* L.). *PLoS ONE* **9**, e85543. (doi:10.1371/journal.pone.0085543)
 17. Millot S, Cerqueira M, Castanheira MF, Øverli Ø, Martins CIM, Oliveira RF. 2014 Use of conditioned place preference/avoidance tests to assess affective states in fish. *Appl. Anim. Behav. Sci.* **154**, 104–111. (doi:10.1016/j.applanim.2014.02.004)
 18. Millot S, Cerqueira M, Castanheira M-F, Øverli Ø, Oliveira RF, Martins CIM. 2014 Behavioural stress responses predict environmental perception in European sea bass *Dicentrarchus labrax*. *PLoS ONE* **9**, e108800. (doi:10.1371/journal.pone.0108800)
 19. Galhardo L, Vitorino A, Oliveira RF. 2012 Social familiarity modulates personality trait in a cichlid fish. *Biol. Lett.* **8**, 936–938. (doi:10.1098/rsbl.2012.0500)
 20. Aires RF, Oliveira GA, Oliveira TF, Ros AFH, Oliveira RF. 2015 Dear enemies elicit lower androgen responses to territorial challenges than unfamiliar intruders in a cichlid fish. *PLoS ONE* **10**, e0137705. (doi:10.1371/journal.pone.0137705)
 21. Carpenter RE, Summers CH. 2009 Learning strategies during fear conditioning. *Neurobiol. Learn. Mem.* **91**, 415–423. (doi:10.1016/j.nlm.2009.01.009)
 22. Vindas MA *et al.* 2012 Omission of expected reward agitates Atlantic salmon (*Salmo salar*). *Anim. Cogn.* **15**, 903–911. (doi:10.1007/s10071-012-0517-7)
 23. Venkatesh B, Erdmann MV, Brenner S. 2001 Molecular synapomorphies resolve evolutionary relationships of extant jawed vertebrates. *Proc. Natl Acad. Sci. USA* **98**, 11 382–11 387. (doi:10.1073/pnas.201415598)
 24. Broglio C *et al.* 2005 Hallmarks of a common forebrain vertebrate plan: specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. *Brain Res. Bull.* **66**, 277–281. (doi:10.1016/j.brainresbull.2005.03.021)
 25. Ganz J, Kaslin J, Freudenreich D, Machate A, Geffarth M, Brand M. 2012 Subdivisions of the adult zebrafish subpallium by molecular marker analysis. *J. Comp. Neurol.* **520**, 633–655. (doi:10.1002/cne.22757)
 26. Ganz J *et al.* 2015 Subdivisions of the adult zebrafish pallium based on molecular marker analysis. *F1000Res.* **3**, 308. (doi:10.12688/f1000research.5595.2)
 27. Teles MC, Almeida O, Lopes JS, Oliveira RF. 2015 Social interactions elicit rapid shifts in functional connectivity in the social decision-making network of zebrafish. *Proc. R. Soc. B* **282**, 20151099. (doi:10.1098/rspb.2015.1099)
 28. Cerda-Reverter JM, Zanuy S, Munoz-Cueto JA. 2001 Cytoarchitectonic study of the brain of a perciform species, the sea bass (*Dicentrarchus labrax*). I. The telencephalon. *J. Morphol.* **247**, 217–228. (doi:10.1002/1097-4687(200103)247:3<217::AID-JMOR1013>3.0.CO;2-U)
 29. Livak K, Schmittgen T. 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2-DeltaDeltaCT method. *Methods* **25**, 402–408. (doi:10.1006/meth.2001.1262)
 30. Lovallo WR. 2005 *Stress & health: biological and psychological interactions*. Thousand Oaks, CA: SAGE Publications.
 31. Sapolsky R. 2004 *Why zebras don't get ulcers*. New York, NY: Henry Holt and Company.
 32. Gliner JA. 1972 Predictable vs. unpredictable shock: preference behavior and stomach ulceration. *Physiol. Behav.* **9**, 693–698. (doi:10.1016/0031-9384(72)90036-4)
 33. Hymowitz N. 1979 Suppression of responding during signaled and unsignaled shock. *Psychol. Bull.* **86**, 175–190. (doi:10.1037/0033-2909.86.1.175)
 34. Guile MN, McCutcheon NB. 1984 Effects of naltrexone and signaling inescapable electric shock on nociception and gastric lesions in rats. *Behav. Neurosci.* **98**, 695–702. (doi:10.1037/0735-7044.98.4.695)
 35. Mormede P, Dantzer R, Michaud B, Kelley KW, Le Moal M. 1988 Influence of stressor predictability and behavioral control on lymphocyte reactivity, antibody responses and neuroendocrine activation in rats. *Physiol. Behav.* **43**, 577–583. (doi:10.1016/0031-9384(88)90211-9)
 36. Tyler K, Moriceau S, Sullivan RM, Meerveld BG-V. 2007 Long-term colonic hypersensitivity in adult rats induced by neonatal unpredictable vs predictable shock. *Neurogastroenterol. Motil.* **19**, 761–768. (doi:10.1111/j.1365-2982.2007.00955.x)
 37. Greiveldinger L, Veissier I, Boissy A. 2007 Emotional experience in sheep: predictability of a sudden event lowers subsequent emotional responses. *Physiol. Behav.* **92**, 675–683. (doi:10.1016/j.physbeh.2007.05.012)
 38. Dess NK *et al.* 1983 Immediate and proactive effects of controllability and predictability on plasma cortisol responses to shocks in dogs. *Behav. Neurosci.* **97**, 1005–1016. (doi:10.1037/0735-7044.97.6.1005)
 39. Thomas R. 2010 Predictability in an unpredictable environment: training the police horse using learning theory. *J. Vet. Behav.* **5**, 218. (doi:10.1016/j.jveb.2009.09.040)
 40. Carlstead K. 1986 Predictability of feeding: its effect on agonistic behaviour and growth in grower pigs. *Appl. Anim. Behav. Sci.* **16**, 25–38. (doi:10.1016/0168-1591(86)90037-7)
 41. Bauer CM, Glassman LW, Cyr NE, Romero LM. 2011 Effects of predictable and unpredictable food restriction on the stress response in molting and non-molting European starlings (*Sturnus vulgaris*). *Comp. Biochem. Physiol. A, Mol. Integr. Physiol.* **160**, 390–399. (doi:10.1016/j.cbpa.2011.07.009)
 42. Arabian JM, Desiderato O. 1975 Preference for signaled shock: a test of two hypotheses. *Anim. Learn. Behav.* **3**, 191–195. (doi:10.3758/BF03213429)
 43. Lockard JS. 1963 Choice of a warning signal or no warning signal in an unavoidable shock situation. *J. Comp. Physiol. Psychol.* **56**, 526–530. (doi:10.1037/h0041552)
 44. Miller RR, Greco C, Vigorito M, Marlin NA. 1983 Signaled tailshock is perceived as similar to a stronger unsignaled tailshock: implications for a functional analysis of classical conditioning. *J. Exp. Psychol. Anim. Behav. Process.* **9**, 105–131. (doi:10.1037/0097-7403.9.2.105)
 45. Belova MA, Paton JJ, Morrison SE, Salzman CD. 2007 Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. *Neuron* **55**, 970–984. (doi:10.1016/j.neuron.2007.08.004)
 46. Cabib S, Puglisi-Allegra S. 2012 The mesoaccumbens dopamine in coping with stress. *Neurosci. Biobehav. Rev.* **36**, 79–89. (doi:10.1016/j.neubiorev.2011.04.012)
 47. Maier SF, Watkins LR. 2010 Role of the medial prefrontal cortex in coping and resilience. *Brain Res.* **1355**, 52–60. (doi:10.1016/j.brainres.2010.08.039)
 48. Pruessner JC *et al.* 2008 Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. *Biol. Psychiatry.* **63**, 234–240. (doi:10.1016/j.biopsych.2007.04.041)
 49. Panula P *et al.* 2010 The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. *Neurobiol. Dis.* **40**, 46–57. (doi:10.1016/j.nbd.2010.05.010)
 50. Tay TL, Ronneberger O, Ryu S, Nitschke R, Driever W. 2011 Comprehensive catecholaminergic projectome analysis reveals single-neuron integration of zebrafish ascending and descending dopaminergic systems. *Nat. Commun.* **2**, 171.
 51. Yamamoto K, Vernier P. 2011 The evolution of dopamine systems in chordates. *Front. Neuroanat.* **5**, 21. (doi:10.3389/fnana.2011.00021)
 52. Davis M. 2000 The role of the amygdala in conditioned and unconditioned fear and anxiety. In *The amygdala: a functional analysis* (ed J Aggleton), pp. 213–287. Oxford, UK: Oxford University Press.
 53. LeDoux JE. 2000 Emotion circuits in the brain. *Annu. Rev. Neurosci.* **23**, 155–184. (doi:10.1146/annurev.neuro.23.1.155)
 54. Folgueira M, Anadon R, Yanez J. 2004 An experimental study of the connections of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). I: Olfactory bulb and ventral area. *J. Comp. Neurol.* **480**, 180–203. (doi:10.1002/cne.20340)
 55. Folgueira M, Anadon R, Yanez J. 2004 Experimental study of the connections of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). II: Dorsal area and preoptic region. *J. Comp. Neurol.* **480**, 204–233. (doi:10.1002/cne.20341)
 56. Striedter GF. 1991 Auditory, electrosensory, and mechanosensory lateral line pathways through the forebrain in channel catfishes. *J. Comp. Neurol.* **312**, 311–331. (doi:10.1002/cne.903120213)

57. Yamamoto N, Ito H. 2005 Fiber connections of the anterior preglomerular nucleus in cyprinids with notes on telencephalic connections of the preglomerular complex. *J. Comp. Neurol.* **491**, 212–233. (doi:10.1002/cne.20681)
58. Yamamoto N, Ito H. 2008 Visual, lateral line, and auditory ascending pathways to the dorsal telencephalic area through the rostralateral region of the lateral preglomerular nucleus in cyprinids. *J. Comp. Neurol.* **508**, 615–647. (doi:10.1002/cne.21717)
59. Braford MR J. 1995 Comparative aspects of forebrain organization in the ray-finned fishes: touchstones or not? *Brain Behav. Evol.* **46**, 259–274. (doi:10.1159/000113278)
60. Echteler SM, Saidel WM. 1981 Forebrain connections in the goldfish support telencephalic homologies with land vertebrates. *Science* **212**, 683–685. (doi:10.1126/science.6971493)
61. Murakami T, Morita Y, Ito H. 1983 Extrinsic and intrinsic fiber connections of the telencephalon in a teleost, *Sebastiscus marmoratus*. *J. Comp. Neurol.* **216**, 115–131. (doi:10.1002/cne.902160202)
62. Portavella M, Vargas JP, Torres B, Salas C. 2002 The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. *Brain Res. Bull.* **57**, 397–399. (doi:10.1016/S0361-9230(01)00699-2)
63. Maximino C, Lima MG, Oliveira KR, Batista Ede J, Herculano AM. 2013 'Limbic associative' and 'autonomic' amygdala in teleosts: a review of the evidence. *J. Chem. Neuroanat.* **48–49**, 1–13. (doi:10.1016/j.jchemneu.2012.10.001)
64. Eichenbaum H, Dudchenko P, Wood E, Shapiro M, Tanila H. 1999 The hippocampus, memory, and place cells: is it spatial memory or a memory space? *Neuron* **23**, 209–226. (doi:10.1016/S0896-6273(00)80773-4)
65. Rodríguez F, Lopez JC, Vargas JP, Gomez Y, Broglio C, Salas C. 2002 Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *J. Neurosci.* **22**, 2894–2903. (doi:10.1523/JNEUROSCI.22-07-02894.2002)
66. Demski LS. 2013 The pallium and mind/behavior relationships in teleost fishes. *Brain Behav. Evol.* **82**, 31–44. (doi:10.1159/000351994)
67. Broglio C *et al.* 2015 Hippocampal pallium and map-like memories through vertebrate evolution. *J. Behav. Brain Sci.* **5**, 109–120. (doi:10.4236/jbbs.2015.53011)
68. Bingman VP, Rodríguez F, Salas C. 2017 The hippocampus of nonmammalian vertebrates. *Evol. Nervous Syst.* **1**(2nd ed), 479–489. (doi:10.1016/B978-0-12-804042-3.00013-0)
69. Ramamoorthi K *et al.* 2011 Npas4 regulates a transcriptional program in CA3 required for contextual memory formation. *Science* **334**, 1669–1675. Epub 2011/12/24. (doi:10.1126/science.1208049)
70. Weng FJ *et al.* 2018 Npas4 is a critical regulator of learning-induced plasticity at mossy fiber-CA3 synapses during contextual memory formation. *Neuron* **97**, 1137–1152.e5. Epub 2018/02/13. (doi:10.1016/j.neuron.2018.01.026)
71. Slattery DA, Morrow JA, Hudson AL, Hill DR, Nutt DJ, Henry B. 2005 Comparison of alterations in c-fos and Egr-1 (zif268) expression throughout the rat brain following acute administration of different classes of antidepressant compounds. *Neuropsychopharmacology* **30**, 1278–1287. Epub 2005/04/07. (doi:10.1038/sj.npp.1300717)
72. Kovacs KJ. 2008 Measurement of immediate-early gene activation: c-fos and beyond. *J. Neuroendocrinol.* **20**, 665–672. Epub 2008/07/08. (doi:10.1111/j.1365-2826.2008.01734.x)
73. Poirier R *et al.* 2008 Distinct functions of Egr gene family members in cognitive processes. *Front. Neurosci.* **2**, 47–55. (doi:10.3389/neuro.01.002.2008)
74. Bozon B, Davis S, Laroche S. 2003 A requirement for the immediate early gene zif268 in reconsolidation of recognition memory after retrieval. *Neuron* **40**, 695–701. (doi:10.1016/S0896-6273(03)00674-3)
75. Jones MW *et al.* 2001 A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nat. Neurosci.* **4**, 289–296. (doi:10.1038/85138)
76. Penke Z *et al.* 2014 Zif268/Egr1 gain of function facilitates hippocampal synaptic plasticity and long-term spatial recognition memory. *Phil. Trans. R. Soc. B* **369**, 20130159. (doi:10.1098/rstb.2013.0159)
77. Bath KG, Schilit A, Lee FS. 2013 Stress effects on BDNF expression: effects of age, sex, and form of stress. *Neuroscience* **239**, 149–156. (doi:10.1016/j.neuroscience.2013.01.074)
78. Marmigere F, Givalois L, Rage F, Arancibia S, Tapia-Arancibia L. 2003 Rapid induction of BDNF expression in the hippocampus during immobilization stress challenge in adult rats. *Hippocampus* **13**, 646–655. (doi:10.1002/hipo.10109)
79. Bassett L, Buchanan-Smith HM. 2007 Effects of predictability on the welfare of captive animals. *Appl. Anim. Behav. Sci.* **102**, 223–245. (doi:10.1016/j.applanim.2006.05.029)
80. Gilbert-Norton LB, Leaver LA, Shivik JA. 2009 The effect of randomly altering the time and location of feeding on the behaviour of captive coyotes (*Canis latrans*). *Appl. Animal Behav. Sci.* **120**, 179–185. (doi:10.1016/j.applanim.2009.06.007)
81. Scherer KR. 2001 Appraisal considered as a process of multi-level sequential checking. In *Appraisal processes in emotion: theory, methods, research* (eds KR Scherer, A Schorr, T Johnstone), pp. 92–120. Oxford, UK: Oxford University Press.
82. Ellsworth PC, Scherer KR. 2003 Appraisal processes in emotion. In *Handbook of affective sciences* (eds RJ Davidson, KR Scherer, HH Goldsmith), pp. 572–595. New York, NY: Oxford University Press.
83. Cerqueira M, Rey S, Silva T, Featherstone Z, Crumlish M, MacKenzie S. 2016 Thermal preference predicts animal personality in Nile tilapia *Oreochromis niloticus*. *J. Anim. Ecol.* **85**, 1389–1400. (doi:10.1111/1365-2656.12555)