Optimistic and pessimistic cognitive judgement bias modulates the stress response and cancer progression in zebrafish

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Abstract

Cognitive judgement bias in decision-making under ambiguity occurs both in animals and humans, with some individuals interpreting ambiguous stimulus as positive (optimism) and others as negative (pessimism). We hypothesize that judgement bias is a personality trait and that individuals with a pessimistic bias would be more reactive to stressors and therefore more susceptible to stress-related diseases than optimistic ones. Here, we show that zebrafish judgment bias is a consistent behavioral trait over time, and that pessimistic and optimistic fish express phenotype-specific neurogenomic responses to stress. Furthermore, both phenotypes show differential activation of the hypothalamic-pituitary-interrenal axis in response to chronic stress, suggesting that optimists have a lower stress reactivity. Accordingly, optimists seem to be more resilient to disease than pessimists, as shown by a lower tumorigenesis in a zebrafish melanoma line [Tg(mtifa:HRAS-GFP)]. Together these results indicate that judgement bias is paralleled by differences in the stress response with implications for disease resilience.

Keywords

cognitive bias; optimism; pessimism; stress reactivity; cancer progression; zebrafish

Introduction

Cognitive biases in decision-making have been extensively documented both in animals and humans. A well-studied bias in animal behavior is judgement bias in decision-making under ambiguity, with some individuals interpreting ambiguous stimulus as positive (optimism) and others as negative (pessimism) [1, 2]. There has been a great interest in the study of animal judgment bias under ambiguity to assess animal affective states, based on the principle that negative affective states induce pessimistic bias. Experimental paradigms to assess judgment bias have thus been developed for a broad range of species in recent years [e.g. 3,4], based on the evaluation of an ambiguous stimulus by the animals. These studies have been mainly focused on the modulation of judgment bias by situational or contextual factors (e.g. environmental enrichment) that are expected to affect the affective state of the animals. Judgment bias has therefore been traditionally considered as a transient condition (i.e. a state). However, despite its response to current affective state, it is possible that cognitive judgment bias can also be consistent within individuals (i.e. optimistic vs. pessimistic phenotypes), in which case it may be also considered as a personality trait [5]. Although a number of studies have shown consistency of individual differences in judgement bias across repeated testing [6 - 8], to the best of our knowledge, no research studies have comprehensively addressed whether judgment bias may reflect a trait, which may be partially due to the conceptual and methodological difficulties inherent to the field of animal personality. However, several tools that were previously developed in the psychological literature and overcome these difficulties have been recapitulated in an integrative theoretical framework [9], which offer a robust and unified approach for the study of personality traits in animals. This framework is mainly based on the analysis of repeatability and several types of the validity (i.e. degree to which a test measures the targeted trait) of the tests that are used to measure the trait of interest.

The hypothesis that judgment bias may reflect a consistent behavioral trait is rather relevant, since it may impact the way individuals respond to and are affected by exposure to chronic stress. In this scenario, it is expected that individuals with a pessimistic bias trait would be more reactive to stressors, because they would consistently interpret ambiguous stimuli as negative, hence over activating the stress response and becoming more susceptible

55 to stress-related diseases than optimistic ones. In fact, patients suffering from stress related disorders often exhibit
56 a pessimistic judgment bias, which frequently lead them to a negative state [10].

57 Here, we used zebrafish (*Danio rerio*), an emerging model organism in neuroscience and biomedicine [11,
58 12] to assess the occurrence of consistent between-subject judgement bias, that is the occurrence of optimistic
59 and pessimistic phenotypes in the population, and subsequently to test the hypothesis that individuals with a
60 pessimistic bias trait would be more reactive to stressors and therefore more susceptible to stress-related diseases
61 than optimistic ones. For this purpose, we have first assessed the repeatability and validity of judgement bias to
62 establish that judgment bias is a repeatable behavior that reflects subjective expectations towards ambiguous
63 stimuli. Then, we characterized how optimistic and pessimistic individuals perceive stressors and regulate body
64 homeostasis in response to stress, by documenting their changes in forebrain gene expression and in the
65 hypothalamic-pituitary-interrenal axis, respectively, in response to chronic stress. Finally, we tested the
66 hypothesis that optimists are more resilient to stress-related diseases than pessimists. For this purpose, and based
67 on the evidence that stress can facilitate cancer progression by modulating cancer hallmarks that mediate the
68 transformation of normal cells into malignant cells [13], we have used a zebrafish melanoma line
69 [Tg(mtifa:HRAS-GFP)] to assess the effect of optimism/pessimism on tumorigenesis.

70 Results

71 Judgement bias is both a behavioral state and a trait

72 We have phenotyped individual zebrafish for judgement bias using a previously validated test for this
73 species that consists in a Go/No-go task [14, 15]. In brief, fish were trained in a half radial maze to Go to a Positive
74 (P) arm of the maze (rewarded with food) and No-Go to a Negative (N) arm (punished with hand net chase). The
75 P and N arms of the maze were positioned 180° from each other and cued with different color marks (green vs.
76 red), whilst the three ambiguous arms (near positive (NP), ambiguous (A), and near negative (NN)) were
77 positioned at equidistant angles, each separated by 45° between the two reference arms (Fig. 1A). After the
78 training phase the response of fish towards the P and N arms was measured in the absence of the reinforcement
79 cues. After P and N arm testing, the response to the unreinforced ambiguous arm (A) spatially located midway

between the two reference arms (N and P; 90°) and cued with a mixed colored card (i.e. half green, half red) was tested. Since an accurate discrimination performance between stimuli (presumably by a generalization response) in the same experimental setup has already been demonstrated in zebrafish [14], a shorter test phase omitting NP and NN cue testing was implemented in this study (Supplemental Note 1). Seventy-three male zebrafish out of the 80 tested (91.25%) learned the discrimination between the P and N arm maze and were subsequently tested for the A arm maze. The response to the A arm shows a bimodal distribution of the frequency of the Judgement Bias Score (JBS), characterized by a lower modal extreme with optimistic-biased fish and a higher modal extreme with pessimistic-biased fish (Fig. 1B). Given this bimodal distribution we have used the lower (optimists) and the upper (pessimists) quartiles of the JBS-frequency distribution for further analyses. The selection of quartiles in this experimental context, as opposed to other divisions such as thirds, offers an efficient data resolution and effectively captures extreme values within our population.

The Judgement Bias Test was repeated over time in order to assess the consistency of the responses to the ambiguous stimulus. Judgement bias has consistently been shown to be modulated by situational or contextual factors that impact an animal's response to ambiguous stimuli. These external factors include changes in health [16], social dynamics [17], and ageing [14], all of which might inherently compromise trait stability over prolonged periods of time. In fact, a rat study that tested for consistency over 7 weeks obtained a repeatability coefficient for the ambiguous stimulus that although significant, was relatively low ($R_{ambiguous}$= 0.23) [6]. Therefore, to ensure a more comprehensive assessment of trait stability, a three-day testing period was selected (i.e. the test phase was repeated in 3 consecutive days). We believe that shorter durations, as have been established in previous studies [8], allow to capture a robust and reliable representation of the judgement bias trait, minimizing potential confounding effects driven by the above-mentioned external factors. Our results show that the latency to enter the ambiguous arm (A) decreased with repeated testing (i.e. (Test 1 < Test 2 < Test 3; Fig. 1C; Table S1), such that JBS values yielded a decrease in the number of pessimistic responses over time (p = 0.005; Fig. 1D; eta^2 = 0.10, 95% CI = [0.02, 1.00]). It is worth to mention that similar results were found when the whole population (rather than just the upper and lower quartiles) was analyzed (Fig. S1A and S1B Fig; Table S1). Repeatability analysis (i.e. analysis of the intraclass correlation coefficient R) revealed that both measures of

judgment bias (i.e. latency to enter the ambiguous arm and JBS) were significantly repeatable when the lower and upper quartiles were considered for the analysis (latency to enter the A arm: R = 0.642; [0.449, 0.773], $p < 0.001$, Fig. 1E; JBS: R = 0.512, [0.283, 0.67], $p < 0.001$, Fig. 1F). For the whole population, repeatability of the behavioral measures of judgment bias, was lower but still significant (latency to enter A: R = 0.43, [0.274, 0.568], $p < 0.001$, Fig. S1C; JBS: R = 0.368; [0.216, 0.499], $p < 0.001$, Fig. S1D). These data indicates that more extreme judgement bias phenotypes are more consistent. It is worth noting that, as a result of our specific protocol settings, a ceiling effect is observed at the upper extreme of our data, specifically in Test 1. A ceiling effect is more likely to impact extreme values, which suggests that this effect may constrain the variability of JBS within this range and potentially influence the intraclass correlation coefficients. However, due to an apparent decrease in the neophobic response towards the ambiguous stimulus in Test 2 and Test 3 (which evoke a general decrease in the JBS), the ceiling effect becomes residual in such tests, with minimal impact on the intraclass correlation coefficients. In other words, this decrease in neophobic response leads to a broader distribution of the higher JBS scores in Tests 2 and 3, reducing the influence of the ceiling effect on the overall variability of the data and, consequently, on the intraclass correlation coefficients.

The fact that optimism increases with repeated testing for judgement bias indicates that individuals are progressively getting familiar with the A arm, hence decreasing a neophobic response towards it. This result is in line with the common view of judgment bias as a measure of affective state. However, the intra-individual consistency of judgement bias along time, as revealed by the positive correlations of JBS across the 3 days of testing, indicates that it is also a consistent behavioral trait, with individuals keeping their relative expression of the trait within the distribution of the trait in the population.

Judgement bias clusters with boldness-related behaviors

The individuals screened (n = 73) and selected (n = 17 per experimental group) for judgement bias were also phenotyped for two established animal personality traits: (1) exploratory behavior, using the open field test (OFT) [18] (Fig. 1G); and (2) boldness, using the novel object test (NOT) [19], in which a novel object (a marble) was introduced into the tank (Fig. 1H). Pessimistic fish exhibited higher values for most behavioral measures of

exploratory behavior than optimistic fish (Supplemental Note 1, Fig. S2A–S2E, and Table S2). In contrast, optimistic fish approached more frequently and spent more time in the novel object zone ($p < 0.001$ in both cases; Supplemental Note 1, Fig. S2F–2J, and Table S2), suggesting that they have a higher motivation to investigate novel objects (aka boldness) than pessimistic fish.

To assess the phenotypic architecture of judgment bias we performed a principal component analysis (PCA) based on the correlation matrix between the 11 behavioral measures extracted from the 3 above-mentioned behavioral assays (Judgement Bias Test, OFT and NOT). Four principal components (PC) were extracted (cumulative variance explained of 82.7%; Fig. 1I and Table 1): PC1 showed a strong loading of mobility, edge-orienting, spatial preference and spatial examination measured in the OFT, suggesting the occurrence of an exploratory behavior module; PC2 showed a strong loading of JBS as well as of novel object examination measured in the NOT, indicating the occurrence of a boldness behavioral module which includes judgment bias; PC3 showed a robust loading of mobility measured in both OFT and NOT, suggesting the occurrence of a general locomotor/activity module; no additional behavioral modules were identified in PC4. Using the first two components of the PCA to combine all metrics load on PC1 and PC2 in a common behavioral parameter space (Fig. 1J), shows that optimistic individuals are behaviorally differentiable from pessimistic ones. Taken together, these results indicate that the JBS clusters with novel object investigation, thereby establishing a link between the response to ambiguous stimuli and the willingness to take risks.

Genomic brain states of judgment bias phenotypes respond differentially to chronic stress

Given the consistency of judgement bias we tested if the optimistic and pessimistic behavioral phenotypes exhibit different brain states, as measured by the transcriptome profiles of the telencephalon and diencephalon, which are key brain areas for cognitive functioning and physiology and behavior regulation, respectively, and how these brain states respond to stress. Individuals were therefore exposed to a validated unpredictable chronic stress (UCS) protocol for zebrafish during 1 month, and a comprehensive transcriptome analysis, using RNA-seq,

157 of the telencephalon and diencephalon was performed at the end of the experiment using for each experimental
158 treatment (n = 5 fish per experimental treatment: control optimists, control pessimists, stressed optimists, and
159 stressed pessimists stress). The number of animals used in our RNA-seq experiment is consistent with that
160 employed in studies addressing neurogenomic responses in zebrafish [20].

161 In control conditions there were 21 differentially expressed (DE) genes between optimists and pessimists
162 in the telencephalon and 5 in the diencephalon, whereas after exposure to chronic stress DE genes were 43 and 2,
163 respectively (Fig. S3A–3F; Table S3 and S4). These results suggest a low differential gene expression between
164 the two non-stressed phenotypes, particularly in the diencephalon. In contrast, the transcriptomic response to
165 chronic stress within each phenotype was more pronounced (Fig. 2A–2F). In response to stress optimists have a
166 higher transcriptomic response in the diencephalon (33 and 50 DE genes between control and stressed conditions
167 in the telencephalon and diencephalon, respectively; Fig. 2A and 2C; Table S3 and S4), whereas pessimists have
168 a small response in the diencephalon and a higher response in the telencephalon (48 and 10 DE genes between
169 control and stressed conditions in the telencephalon and diencephalon, respectively; Fig. 2B and 2D).
170 Interestingly, there was almost no overlap in the lists of DE expressed genes in response to stress between
171 optimists and pessimists in either the telencephalon or the diencephalon (Fig. 2E). Looking into the total number
172 of transcripts that were either up- or down-regulated in a phenotype, optimistic fish under stressed conditions had
173 more transcripts down-regulated and fewer transcripts up-regulated in the diencephalon, whereas their pessimistic
174 counterparts had the opposite pattern with fewer transcripts down-regulated and more transcripts up-regulated
175 (Fig. 2F). In the telencephalon, the overall pattern of directional change of pessimists was similar to that of
176 optimists. Taken together, these results indicate that the two judgement bias phenotypes show different brain
177 transcriptomic responses to chronic stress, suggesting differences in cognitive processing of stressors (in the
178 telencephalon) as well as in the regulation of body homeostasis and behavior (in the diencephalon). In fact, the
179 hierarchical clustering of these comparisons (i.e. optimistic control versus optimistic stressed and pessimistic
180 control versus pessimistic stressed; Fig. 2A–2D) indicated that all individuals from each treatment (control vs.
181 stress) were grouped together in separate clusters, reflecting a high consistency of their transcriptome profiles.
182 On the contrary, a consistent clustering was rarely observed in the comparisons between optimists and pessimists

183 either in control or stressed conditions (Fig. S3A–3D). Finally, Gene Ontology (GO) analysis of DE genes
184 detected overrepresentation of specific biological process, molecular processes and cellular component GO terms
185 according to cognitive bias phenotype (Supplemental Note 2, Table S5 and S6).

186 In summary, the observed divergence in stress-driven changes in neurogenomics states between optimists
187 and pessimists suggests that these two behavioral phenotypes have different responses to stress.

188

189 Judgement bias modulates the response to chronic stress

190 Given the differences in brain transcriptome responses to stress described above, we tested whether
191 differences in judgment bias may be associated to inter-individual variation in the stress response at different
192 levels of the hypothalamic-pituitary-interrenal (HPI) axis. Therefore, using the same experimental protocol, we
193 also assessed the consequences of chronic stress in optimistic and pessimistic zebrafish at the end of the
194 experiment by measuring cortisol levels under control and stressed conditions as well as expression levels of key
195 genes related to the HPI axis in the brain (i.e. telencephalon and diencephalon), namely corticotropin releasing
196 hormone (*crh*), and glucocorticoid (*gr*) and mineralocorticoid (*mr*) receptors. We also calculated the *mr/gr* ratio,
197 which is a key factor in the regulation of stress reactivity [21].

198 Chronic stress had a residual main effect (p = 0.058; Table S7) on cortisol levels, with stressed fish
199 showing lower cortisol levels than control fish. Indeed, planed comparisons revealed that control optimists and
200 pessimists have similar levels of circulating cortisol, but pessimists decrease their cortisol levels in response to
201 stress (p = 0.036) whereas optimists do not (Fig. 3A). Stressed fish, irrespective of phenotype, also displayed
202 higher levels of *crh* transcript levels at both the telencephalon (p = 0.052) and diencephalon (p = 0.014) (Fig. 3B
203 and 3F). Regarding the expression of *gr* and *mr* receptors in the telencephalon, there was an interaction effect
204 between phenotype (optimists vs. pessimists) and treatment (p = 0.008 and p = 0.005, respectively). In control
205 conditions, optimists have higher levels of expression of both receptor types than pessimists (p = 0.015 and p =
206 0.001, respectively), resulting also in a higher *mr/gr* ratio in optimists (p = 0.003) (Fig. 3C–3E). When exposed
207 to stress, optimists decreased *mr* levels (p = 0.027), resulting in a significant decrease of their *mr/gr* ratio (p =
208 0.034). In contrast, in response to stress, pessimists showed a significant increase in *gr* levels (p = 0.022), and a

209 non-significant trend to increased *mr* levels ($p = 0.096$), resulting in unchanged *mr/gr* ratio in comparison to
210 control conditions. As a result, after exposure to chronic stress there were no significant differences between
211 optimists and pessimists on their expression levels of *gr*, *mr* or the *mr/gr* ratio. Notably, phenotype had a
212 significant main effect ($p = 0.003$) on the *mr/gr* ratio.

213 Regarding the expression of *gr* and *mr* receptors in the diencephalon, there are no significant differences
214 between optimists and pessimists in control conditions, but the *mr/gr* ratio is higher in optimists ($p = 0.0014$)
215 (Fig. 3G–3I; Table S7). When exposed to stress optimists showed no significant changes in either *gr* or *mr*
216 receptor levels, but they showed a non-significant trend for decreased *mr/gr* ratio ($p = 0.056$), whereas pessimists
217 showed increase *gr* levels ($p = 0.011$), but no significant changes in either *mr* or *mr/gr* ratio. As a result, after
218 exposure to chronic stress *gr* levels are significantly lower in optimists than in pessimists ($p = 0.015$), but there
219 were no significant differences between them in either *mr* levels or *mr/gr* ratio. Notably, phenotype had a
220 significant main effect ($p < 0.001$) for the *mr/gr* ratio.

221 The higher *mr/gr* ratios detected in optimists both in the telencephalon and diencephalon may protect them
222 against chronic stress, hence promoting resilience to disease.

223

224 Judgement bias modulates cancer progression

225 Given the differential response of optimistic and pessimistic zebrafish to chronic stress, we decided to test
226 if it translates into differential susceptibility/ resilience to disease. Because optimists seem to have a lower stress
227 reactivity, we hypothesized that they should have a lower allostatic load, hence being more resilient to stress-
228 induced disease than pessimists. Based on the known association between stress and cancer we decided to test
229 this hypothesis using a zebrafish melanoma model (mitfa:HRAS) that exhibits full penetrance by 3 months of age
230 [22]. Transgenic (mitfa:HRAS^{G12V}-GFP) zebrafish and their WT siblings were phenotyped for judgement bias
231 between 44 and 66 days post hatching (dph), before melanoma onset, using our previously validated protocol [14,
232 15]. Subsequently, transgenic fish were exposed to chronic stress. Half the optimists and half the pessimists were
233 therefore exposed to the unpredictable chronic stress protocol for 1 month, and the other half of each phenotype

234 remained undisturbed (control group) to assess the effect of stress on tumorigenesis. Individuals were checked
235 weekly for the occurrence of melanoma.
236 Tg(mitfa:HRAS^{G12V}-GFP) individuals (Fig. 4A) are more pessimistic than their WT siblings (Table S8), even
237 before the first signs of melanoma (44-66 dph), as shown by transgenic fish exhibiting increased latencies to enter
238 the ambiguous arm ($p < 0.001$; Fig. 4B) and having higher JBS values ($p < 0.001$; Fig. 4C; Cohen's d = -0.92,
239 95% CI = [-1.45, -0.40]). The first tumor incidences were recorded on week 9, which matches previously
240 described tumor onset on this line [23]. Interestingly, in undisturbed fish the cumulative percentage of tumor
241 incidence is significantly lower in optimists than in pessimists at weeks 9 and 10 ($p = 0.043$ and $p = 0.043$,
242 respectively; Fig. 4D), indicating an earlier onset of melanoma in pessimistic individuals (Table S9 and S10).
243 Chronic stress increases cumulative percentage of tumor incidence in optimists ($p < 0.001$) but had no effect on
244 pessimists ($p = 0.15$), which already had a high cumulative percentage in undisturbed conditions, resulting in no
245 difference between optimists and pessimists under chronic stress. At 98 dph all individuals were euthanized and
246 tumor fraction, and PCNA positive tumor cells were quantified. Pessimistic, but not optimistic, individuals
247 showed an increase in tumor fraction in response to chronic stress (Fig. 4E; Table S11), with pessimists showing
248 already a higher level of tumor cell proliferation than optimists, as measured by PCNA, before exposure to stress
249 (Fig. 4F; Table S11).
250 The fact that pessimists show a higher susceptibility to tumorigenesis and higher levels of tumor cell
251 proliferation than optimists indicate that optimistic individuals are more resilient to disease than the pessimistic
252 ones.

255 Discussion

256 Our results indicate that judgement bias, usually considered as a behavioral state, can also be seen as a
257 trait (i.e. individually consistent over time), that is correlated with boldness-related behaviors. When exposed to
258 chronic stress pessimistic and optimistic fish presented different neuromolecular responses, as measured by
259 transcriptome changes in the telencephalon and diencephalon, suggesting that they perceive stressors and regulate
260 body homeostasis in response to stress differently. Furthermore, optimists show higher mineralocorticoid
261 receptor/ glucocorticoid receptor ratios in both brain areas, and other differences in the hypothalamic-pituitary-

interrenal axis that suggest that they have a lower stress reactivity. Accordingly, optimists seemed to be more resilient to disease than pessimists, as shown by a lower tumorigenesis of optimists in a zebrafish melanoma line [Tg(mtifa:HRAS-GFP)]. Together these results indicate that cognitive bias is a personality trait paralleled by differences in the stress response with implications for disease resilience.

Judgement bias in animals has been seen as a state variable that indicates the affective state of the individuals, and has been widely used in animal welfare studies (i.e. pessimistic bias seen as reporting negative affective state and poor welfare). The occurrence of judgement bias as a trait, that is as a relatively stable behavioral feature of the individual over time (aka personality trait), requires showing significant repeatability across repeated tests over time [24, 25], as well as the establishment of several types of test validity [9]. In this study, we established the repeatability of judgement bias, as well as its ecological validity (i.e. the relationship between the targeted trait and other ecologically important traits). Interestingly, the distribution of JBS in the population shows a bimodal distribution suggesting disruptive selection for the evolution of alternative phenotypes in this judgement bias dimension. Together, these results indicate that judgment bias can be seen as a trait. Furthermore, our PCA analysis indicates that the response to the ambiguous stimulus in the judgement bias test is correlated with the response to the novel object test, positively linking the response to an ambiguous stimulus with the willingness to take risks. A similar link between boldness-related traits and judgment bias has already been reported in a recent study, in which dogs that were rated as more prone to non-social-fear were also more likely to display an optimistic response [26].

The establishment of judgement bias as a personality trait raises the possibility that individuals with different judgement bias phenotypes (i.e. optimists/pessimists) may respond differently to stressors, and therefore express differential resilience to stress-related diseases. We have tested the hypothesis that optimists and pessimists perceive and respond differently to stress by looking into whole-genome changes in gene expression in two key brain regions for the stress response: (1) the telencephalon, which includes brain associative areas that integrate multimodal sensory stimuli and assess the salience and valence of environmental stimuli [27, 28], and (2) the diencephalon, which regulates bodily responses to stress through the modulation of central peptide systems and neuroendocrine axis [29, 30]. Of the differentially expressed genes in the telencephalon of chronically

288 stressed optimists, some of them were identified to have a role in the mediation of the detrimental effects of
289 chronic stress (e.g. *avp*, *fads2*, *klf11a*) and others in sterol metabolism (i.e. *hsd17b7*, *sqlea*, *sc5d*; Gene Ontology
290 Biological Processes 'Sterol biosynthetic process' and 'Cholesterol metabolic process'), which has also been
291 shown to be dysregulated under stress in other organisms [31]. Differentially expressed genes in the brain of
292 stressed pessimists include a set of genes (e.g. upregulated: *nr1d4a*, *nr1d4b*; downregulated: *apoeb*, *kcnj2a*,
293 *cldn11a*) previously linked to stress in multiple species [32-34]. Notably, claudins (*cldn*) have previously been
294 identified as a molecular biomarker of stress with an impact on health and cognition [35]. The fact that chronic
295 stress induces different profiles of gene expression between optimists and pessimists in both brain regions,
296 indicates phenotype-specific neurogenomic responses to stress at the brain level. It is important to note that these
297 data primarily identify neurogenomic differences between these personality traits and stress responses. However,
298 they do not establish a direct link between judgement bias-related phenotypes and stress reactivity, which
299 underscores the need for future research to investigate potential mediating factors involved in stress resilience. In
300 parallel, the neuroendocrine stress axis (i.e. HPI) also showed differences between the two phenotypes. The
301 expression of *crh* in the diencephalon and cortisol levels were used as indicators of the activity of the HPI axis,
302 whereas the expression of the *gr* and *mr* receptors, provided a measure of the sensitivity of the target tissues
303 (telencephalon and diencephalon) to circulating cortisol. In particular, the *mr*/*gr* ratio has been used as a marker
304 of stress vulnerability, since detrimental effects of chronic stress on cognition seem to be GR-mediated, and
305 unbalanced *mr*/*gr* ratios in the limbic brain have been associated to impaired functioning of the emotional and
306 cognitive systems under stress [21]. Higher *mr*/*gr* ratios are therefore indicative of more resilient responses to
307 chronic stress. In baseline conditions there were no differences in cortisol levels between optimists and pessimists,
308 and in response to chronic stress pessimists, but not optimists, decrease cortisol levels. Given that there were no
309 differences between phenotypes neither changes in response to chronic stress in the expression of *crh*, the stress-
310 driven hypocortisolism observed in pessimists does not seem to be due to a down-regulation of the HPI axis but
311 rather to a stress-induced adrenal insufficiency [36]. This interpretation is based on unexpected findings and may
312 benefit from further investigation, while it is true that decreased cortisol levels have been broadly reported in
313 patients with a post-traumatic stress disorder [37] and also in healthy individuals living under stress conditions

314 [38-40]. In baseline conditions, optimistic fish also exhibit a higher *mr/gr* ratio than pessimists, suggesting the
315 latter may have a higher susceptibility to detrimental consequences of chronic stress. Interestingly, under chronic
316 stress, this ratio decreases in optimists but remains relatively stable in pessimists. This intriguing pattern suggests
317 that while optimistic individuals may have an initial advantage with a higher *mr/gr* ratio, they could also harbor
318 a mechanism to attenuate stress-related elevations in glucocorticoids and mineralocorticoids. Therefore, Mr
319 binding in optimists could prevent excessive Gr binding and its associated stress effects, whilst also decreasing
320 *mr/gr* ratio. In contrast, the stability of the *mr/gr* ratio in pessimists under chronic stress may indicate a higher
321 susceptibility to the detrimental consequences of prolonged stress exposure. These results are in agreement with
322 those found in Arctic charr, in which more reactive individuals were characterized by lower *mr/gr* ratios in
323 comparison to proactive charr showing decreased tolerance and performance under stress [41].

324 Given the known link between stress and cancer, we have assessed differences in tumorigenesis in a
325 melanoma line [Tg(mtifa:HRAS-GFP)] between optimists and pessimists exposed to chronic stress. We found a
326 very significant impact of judgement bias phenotype in tumorigenesis in unstressed individuals, with a higher
327 melanoma incidence at an early age in pessimistic individuals. The exposure to chronic stress has a significant
328 impact in tumorigenesis in optimists, that start from a low melanoma incidence, but not in pessimists that start
329 already from high melanoma incidence levels even before stress exposure. Notably, before exposure to stress
330 pessimists already exhibited higher proliferation of tumor cells than optimists, as revealed by PCNA, and after
331 exposure to stress pessimists also shown more enlarged tumors relative to their body size. The link between stress
332 and cancer has been discussed for decades without solid clinical or epidemiological evidence. However, recent
333 animal studies have shown that stress can facilitate cancer progression by regulating molecular and systemic
334 mechanisms that mediate hallmarks of cancer, such as changes in NK cell proliferation, angiogenesis, tumor
335 vascularization and invasiveness and metastasis [42-48]. Moreover, animal studies have shown that exposure to
336 stress during a critical phase of cancer has a greater impact on its progression than non- synchronized stress
337 episodes [13]. This is particularly relevant because in our experiment exposure to chronic stress overlapped with
338 the known melanoma onset period in the zebrafish line used [23]. Our results confirm the role of stress on

339 tumorigenesis and further indicate that between subject differences can be explained by personality traits, such as
340 the judgement bias phenotypes studied here.

341 In summary, our work highlights the role of psychological factors (i.e. cognitive judgement bias) on the
342 regulation of the brain processing of stress and on the physiological stress response, with an impact in stress-
343 related disease (e.g. cancer). Elucidating how such psychological factors modulate stress vulnerability and its
344 associated disease susceptibility improves our understanding of individual differences in cancer outcomes, and
345 opens the way to conceptualize psychological interventions that might mitigate disease states.

346 Methods

347 Fish and Housing

348 Fish used for this study were 4-5 months old male wild-type zebrafish (*Danio rerio*) of the Tuebingen (TU) line
349 and 1.5-3 months old males of the Tg(mitfa:HRAS^{G12V}-GFP) line originally developed by the Hurlstone
350 Laboratory (Manchester University, U.K.). All procedures were performed in accordance with the relevant
351 guidelines and regulations for animal experimentation.

352 Experiment 1

353 ***Behavioral assays***. Individual zebrafish were first screened in an optimistic – pessimistic dimension by using a
354 previously validated judgment bias test for this species [14, 15, 49]. In brief, a go/no-go task was designed in a
355 half radial maze where individual zebrafish were trained to approach a positive cue (P; food reward) and to avoid
356 a negative cue (N; punishment). Once fish were able to distinguish between P and N cues (as indicated by different
357 latencies to enter each cued arm), their response to an ambiguous (A) cue (an intermediate location/color cue
358 between the P and N locations/color cues) was then tested. In order to assess if judgement bias of the A stimulus
359 of the JBT is associated with other behavioral phenotypes, fish that learned the discrimination between N and P
360 stimuli were selected and tested in an open field test (OFT) and in a novel object test (NOT).

361 ***Unpredictable Chronic Stress (UCS) protocol***. Screened individuals (i.e. optimistic and pessimistic zebrafish)
362 were exposed to a validated UCS protocol for this fish species [50], in which individuals were subjected to a

363 variety of stressors. Each individual experienced two stressors each day, at varying times, for 30 days. All fish of
364 the same experimental tank were given the same stressor at the same time.

365 ***RNA isolation, qPCR and RNA-Sequencing***. Total RNA was isolated from the frozen telencephalon and
366 diencephalon. Isolation of total RNA was performed using the RNeasy® Lipid Tissue Mini Kit (Quiagen),
367 according to the manufacturer's instructions. The first-strand of cDNA was synthesized using iScript™ cDNA
368 Synthesis Kit (Bio-Rad). All assays were run using 384-well optical plates on a QuantStudio™ 7 Flex Real-Time
369 PCR System (Applied Biosystems™, Thermo Fisher). RNA-Seq libraries were prepared according to the Illumina
370 RNA-Seq protocol and sequenced at the Genomics Unit of Instituto Gulbenkian de Ciência using a NextSeq
371 system to generate single-end 75-bp reads.

372 ***Cortisol analysis***. Whole body cortisol levels were used as a proxy for circulating cortisol levels [51]. Cortisol
373 levels were assessed using an enzyme immunoassay (EIA) kit (Cayman Chemical Company) following the
374 manufacturer's instructions.

375 Experiment 2

376 ***Behavioral assay and stress protocol.*** Transgenic zebrafish from 44 to 66 dph (i.e. before melanoma onset [23])
377 were tested for judgment bias and compared with age-matched WT siblings' fish. Based on the JBT, transgenic
378 fish that learned the task were classified in an optimistic/pessimistic dimension before entering a UCS experiment
379 (as described in Experiment 1). Fish showing visible tumor masses before entering the judgment bias screening
380 were discarded from the experiment.

381 ***Tumor assessment.*** Tumor appearance was assessed macroscopically on a weekly basis, from week 9 (66 dph;
382 experiment start day) to week 13 (98 dph; experiment end date). Individuals were scored for the onset of a vertical
383 growth phase (the lesion develops vertically, forming a nodule), and the presence of an outgrowth in any direction
384 was visually monitored.

385 ***Tumor measurements.*** Fish were euthanized the day after the UCS protocol ended. Whole fish were fixed,
386 decalcified, and then processed for paraffin-embedding. Immunohistochemical detection of PCNA was performed
387 using a mouse monoclonal PCNA antibody (PCNA (P10): sc-56; Santa Cruz Biotecnology; dilution 1/100).

PCNA-positive cells were revealed by incubation with 3,3'-diaminobenzidine tetrahydrochloride (Liquid DAB+; Palex). Tumor fraction and PCNA positive tumor cells were scored blindly to phenotype and treatment.

Statistical analyses

The following statistical tests were run in R (v.4.2.2) and SigmaStat (v.3.5). Analysis of the judgment bias testing in WT (Experiment 1) and melanoma fish (Experiment 2) was performed with the R software [52] packages "lme4" [53] and "afex" [54] for the linear mixed effects models (GLMM) and the "emmeans" package [55] for planned comparisons. Generalized linear mixed models (GLMMs) were also used for the analyses of cortisol and stress-related gene expression levels (Experiment 1) and the hallmarks of cancer progression (Experiment 2). A non-parametric two-tailed Kruskal-Wallis test, was used to analyze the JBS in melanoma (Experiment 2) fish. Statistical analyses of the behaviors performed in the OFT and NOT (Experiment 1) were analyzed using a two-way ANOVA followed by an all pairwise multiple comparison procedure (Holm-Sidak's test).

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Supporting information:

Supplement Methods

Supplemental Text

Supplementary Figures and Tables

Fig. S1. Behavioral characterization of judgment bias in zebrafish considering the whole population (n = 73). (A) Performance of male WT zebrafish in the judgement bias paradigm with repeated testing. Different letters indicate significant differences between experimental groups (Test 1, Test 2, and Test 3) for each Treatment (P, A, N) following planned comparisons tests. Data are expressed as mean ± s.e.m. (B) JBS of male WT zebrafish with repeated testing. Different letters indicate significant differences between experimental groups (Test 1, Test 2, and Test 3) following planned comparisons tests. Data are expressed as mean ± s.e.m. (C) Bootstrap repeatabilities for the latency to enter the ambiguous arm of the behavioral apparatus. (D) Bootstrap repeatabilities for the JBS.

Fig. S2. Behavioral differences between fish from the upper and lower quartiles of the Judgment Bias Score (JBS; n = 17 per experimental group: optimists and pessimists): (A) distance moved (cm) in OFT; (B) number of crossings between the inner and outer zones in OFT; (C) time spent in the inner zone (s) in OFT; (D) distance from walls (cm) in OFT; (E) angular velocity (deg/s) in OFT; (F) distance moved (cm) in NOT; (G) latency to first approach to the novel object (s) in NOT; (H) number of approaches to the novel object in NOT; (I) time spent in the novel object zone (s) in NOT; (J) angular velocity (deg/s) in NOT. Asterisks and different letters indicate significant differences between the experimental groups at each sampling point following planned comparisons tests. Data are expressed as mean ± s.e.m.

Fig. S3. Phenotypically driven changes in gene expression in the brain of control and stressed zebrafish (n = 5 per experimental group: optimists control, pessimists control, optimists stress, and pessimists stress). (A) Hierarchical clustering of control individuals from each phenotype (optimists versus pessimists; columns) and of DEG (lines) in the telencephalon; (B) Hierarchical clustering of stressed individuals from each phenotype (optimists versus pessimists; columns) and of DEG (lines) in the telencephalon; (C) Hierarchical clustering of control individuals from each phenotype (optimists versus pessimists; columns) and of DEG (lines) in the diencephalon; (D) Hierarchical clustering of pessimistic stressed from each phenotype (optimists versus pessimists; columns) and of DEG (lines) in the diencephalon. Heatmaps represent normalized gene expression levels (red, high expression; blue, low expression); (E) Venn diagram showing the number of DEG shared between each optimistic group and their pessimistic counterparts (optimists versus pessimists) in the telencephalon and diencephalon; (F) Total number of DEG up- and down-regulated relative to each optimistic group and their stressed counterparts (optimists versus pessimists) in the telencephalon and diencephalon.

Table S1. Results of the general linear mixed model to assess the effects of Test (Test 1 versus Test 2 versus Test 3), Treatment (Positive versus Ambiguous versus Negative), and the double interaction among these variables**.** Partial Eta Squared estimates of effect sizes are given for these factors. *Indicates a significant effect.

Table S2. Results of the two-way ANOVA to assess the effects of Phenotype (Optimists versus Pessimists), Time (2 min versus 4 min versus 6 min versus 8 min versus 10 min), and the double interaction among these variables. Partial Eta Squared estimates of effect sizes are given for these factors. *Indicates a significant effect.

Table S3. List of DEG in the telencephalon.

Table S4 . List of DEG in the diencephalon.

Table S5. Characterization of the DE genes in the telencephalon obtained using ORA for GO, and summarized using GOSlim terms.

Table S6. Characterization of the DE genes in the diencephalon obtained using ORA for GO, and summarized using GOSlim terms.

611 **Table S7.** Results of the general linear mixed model to assess the effects of Phenotype (Optimists versus
612 Pessimists), Treatment (Control versus Stress), and the double interaction among these variables. Partial Eta
613 Squared estimates of effect sizes are given for these factors. *Indicates a significant effect.
614
615 **Table S8.** Results of the general linear mixed model to assess the effects of Genotype (mitfa:HRAS^{G12V}-GFP
616 versus WT), Treatment (Positive versus Ambiguous versus Negative), and the double interaction among these
617 variables. Partial Eta Squared estimates of effect sizes are given for these factors. *Indicates a significant effect.
618
619 **Table S9**. Comparison of the survival curves of the different experimental groups. Treatments were compared
620 using the log-rank test. Correction of p values was performed using the Holm-Sidak method. *Indicates a
621 significant effect.
622
623 **Table S10.** Comparison of the survival probability at specific timepoints between treatments that were
624 significantly different. Note that when the survival curve is zero the statistic is infinite. *Indicates a significant
625 effect.
626
627 **Table S11**. Results of the general linear mixed model to assess the effects of Phenotype (Optimists versus
628 Pessimists), Treatment (Control versus Stress), and the interaction among these variables. Partial Eta Squared
629 estimates of effect sizes are given for these factors. *Indicates a significant effect.
630
631 **Table S12.** Procedure of the unpredictable chronic stress (UCS) protocol in zebrafish.
632
633 **Table S13.** Primer sequences, amplicon lengths and annealing parameters for the genes used in the qPCR.
634
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639 # Figure captions

640 **Fig. 1. Behavioral characterization of judgement bias in zebrafish.** Behavioral characterization of judgment
641 bias in male zebrafish considering the upper and lower quartiles of the Judgment Bias Score (JBS; n = 34). (**A**)
642 Diagram of the half radial maze showing the two reference (positive/rewarded (P) and negative/aversive (N)) and
643 the ambiguous (A) locations that were used in the judgment bias test (JBT) for zebrafish; (**B**) JBS-frequency
644 distribution of the fish that learned the judgment bias task (n = 73); (**C**) Performance of male WT zebrafish in the
645 judgment bias paradigm with repeated testing. Different letters indicate significant differences between the
646 experimental groups (Test 1, Test 2, and Test 3) for each Treatment (P, N, A) following planned comparisons
647 tests. Data are expressed as mean ± s.e.m.; (**D**) JBS of male WT zebrafish with repeated testing. Different letters
648 indicate significant differences between experimental groups (Test 1, Test 2, and Test 3) following planned
649 comparisons tests. Data are expressed as mean ± s.e.m.; (**E**) Bootstrap repeatabilities for the latency to enter the

650 ambiguous arm of the behavioral apparatus; (**F**) Bootstrap repeatabilities for the JBS. (**G**) Diagram of the Open
651 Field Test (OFT); (**H**) Diagram of the Novel Object Test (NOT); (**I**) Degrees of correlation (r) between the
652 different behavioral metrics, illustrated in the cladogram as degrees of association; (**J**) First two components of a
653 Principal Component Analysis (PCA) on all metrics derived from the judgment bias, open field and novel object
654 behavioral tests (JBT, OFT and NOT, respectively), comparing between optimistic and pessimistic fish in a
655 behavioral parameter space. The first two dimensions explain 35.1 and 25.2% of the variance, respectively.
656

657 **Fig. 2. Chronic stress-driven changes in the brain of optimistic and pessimistic zebrafish.** Chronic stress-
658 driven changes in gene expression in the brain of judgment bias-related phenotypes (n = 5 per experimental group:
659 optimists control, pessimists control, optimists stress, and pessimists stress). (**A**) Hierarchical clustering of
660 optimistic individuals from each treatment (control versus stress; columns) and of DEG (lines) in the
661 telencephalon; (**B**) Hierarchical clustering of pessimistic individuals from each treatment (control versus stress;
662 columns) and of DEG (lines) in the telencephalon; (**C**) Hierarchical clustering of optimistic individuals from each
663 treatment (control versus stress; columns) and of DEG (lines) in the diencephalon; (**D**) Hierarchical clustering of
664 pessimistic individuals from each treatment (control versus stress; columns) and of DEG (lines) in the
665 diencephalon. Heatmaps represent normalized gene expression levels (red, high expression; blue, low
666 expression); (**E**) Venn diagram showing the number of DEG shared between each control group and their stressed
667 counterparts (control versus stress) in the telencephalon and diencephalon; (**F**) Total number of DEG up- and
668 down-regulated relative to each control group and their stressed counterparts (control versus stress) in the
669 telencephalon and diencephalon.
670

671 **Fig. 3. Differential levels of HPI axis-related factors between optimistic and pessimistic zebrafish.**
672 Differential levels of a comprehensive set of factors associated with the HPI axis between judgment bias-related
673 phenotypes exposed to the effects of chronic stress (n = 12 per experimental group: optimists control, pessimists
674 control, optimists stress, and pessimists stress). (**A**) Cortisol; (**B**) *crh* expression in the telencephalon; (**C**) *gr*
675 expression in the telencephalon; (**D**) *mr* expression in the telencephalon; (**E**) *mr/gr* ratio in the telencephalon; (**F**)

676 *crh* expression in the diencephalon; (**G**) *gr* expression in the diencephalon; (**H**) *mr* expression in the diencephalon;
677 (**I**) *mr/gr* ratio in the diencephalon. Different letters indicate significant differences between the experimental
678 groups following planned comparisons tests. Data are expressed as mean ± s.e.m.
679
680 **Fig. 4. Differences in onset and progression of melanoma tumor between optimistic and pessimistic**
681 **zebrafish.** Differences in onset and progression of melanoma tumor between judgment bias-related phenotypes
682 exposed to the effects of chronic stress (n = 7-11 per experimental group: optimists control, pessimists control,
683 optimists stress, and pessimists stress). (**A**) Representative image of juvenile transgenic zebrafish harboring
684 melanoma tumor and its corresponding histological section; (**B**) Performance of juvenile melanoma zebrafish and
685 their WT siblings in the judgment bias paradigm (n = 32 each group). Different letters indicate significant
686 differences between the experimental groups (transgenic fish and WT) for each Treatment (P, N, A) following
687 planned comparisons tests. Data are expressed as mean ± s.e.m.; (**C**) JBS of juvenile melanoma zebrafish and
688 their WT siblings. Different letters indicate significant differences between experimental groups (transgenic fish
689 and WT); (**D**) Melanoma occurrence over time in juvenile transgenic fish (OC optimists control; PC pessimists
690 control; OS optimists stress; PC pessimists stress). Asterisks indicate statistically significant differences between
691 the experimental groups at each sampling point; (**E**) Tumor fraction (%) in the different experimental groups of
692 juvenile melanoma fish. Different letters indicate significant differences between the experimental groups
693 following planned comparisons tests. (**F**) PCNA-positive tumor cells (%) in the different experimental groups of
694 juvenile melanoma fish. Different letters indicate significant differences between the experimental groups
695 following planned comparisons tests.
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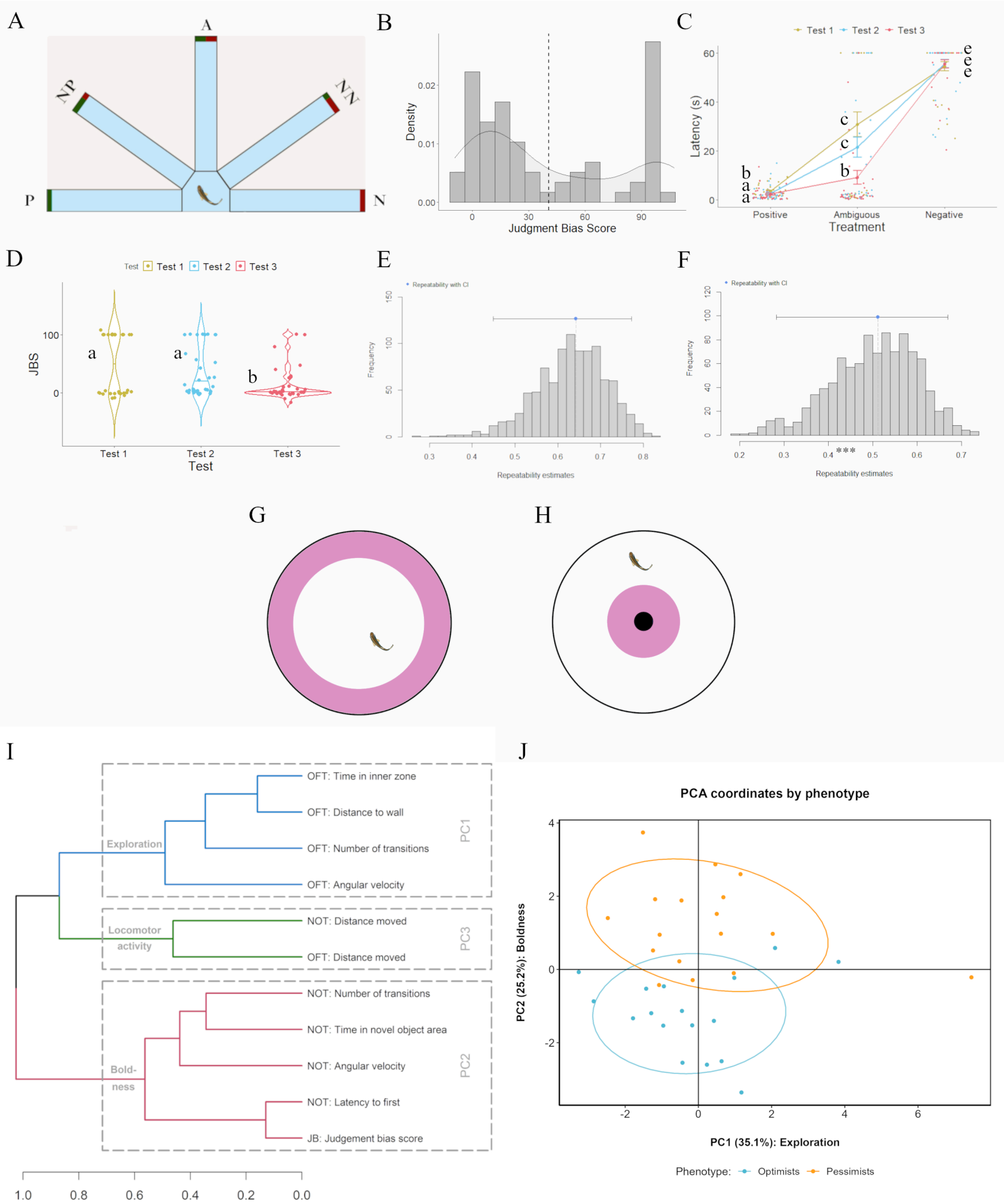
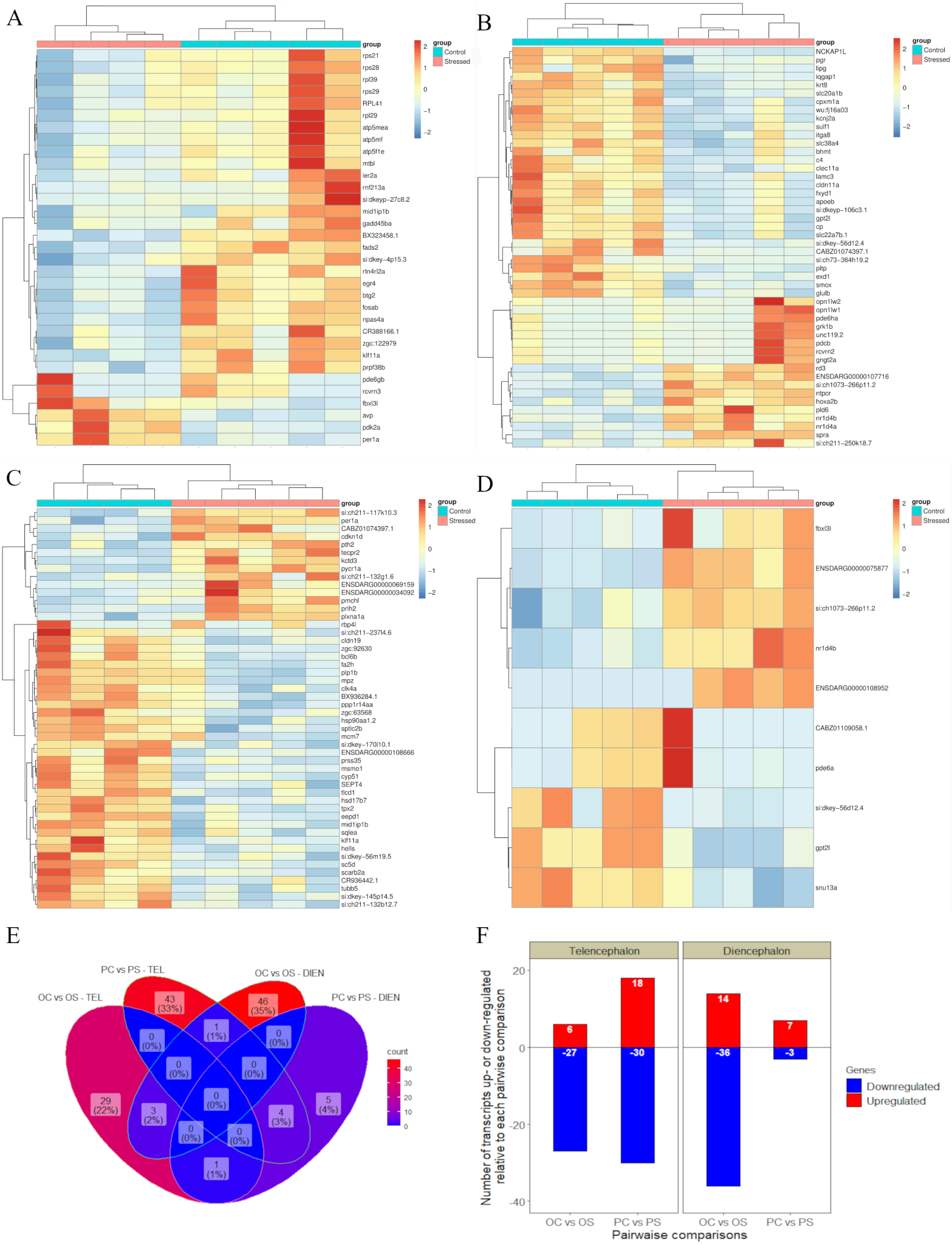
			Principal component loadings [a]			
Test	Behavioral parameter	Biological meaning of the metric	PC1 Exploration	PC2 Boldness	PC3 Locomotor/ activity	PC4
JBT	JBS	Decision-making under ambiguity	0.131	**0.717**	0.081	-0.179
OFT	Mean distance moved	Mobility	-0.571	-0.009	**-0.645**	0.374
	Time spent in the inner zone	Spatial preference	**0.906**	0.264	-0.031	0.017
	Number of crossings between the inner and outer zones	Spatial examination	**0,780**	0.441	-0.282	0.114
	Mean distance from walls	Edge-orienting	**0.885**	0.421	-0.050	0.007
	Mean angular velocity	Mobility	**-0.804**	0.139	0.200	-0.370
NOT	Mean distance moved	Mobility	-0.491	0.409	**-0.651**	-0.157
	Latency to the first approach to the novel object	Willingness to take risks	-0.143	**0.707**	-0.209	-0.162
	Time spent in the novel object zone	Object examination	0.261	-0.594	-0.517	-0.486
	Number of crossings between the novel object and empty zones	Object examination	0.496	**-0.672**	-0.346	-0.319
	Mean angular velocity	Mobility	-0.280	0.548	-0.117	-0.598
		Eigenvalue [b]	3.861	2.750	1.417	1.071
		% Variance explained	35.107	25.007	12.885	9.741

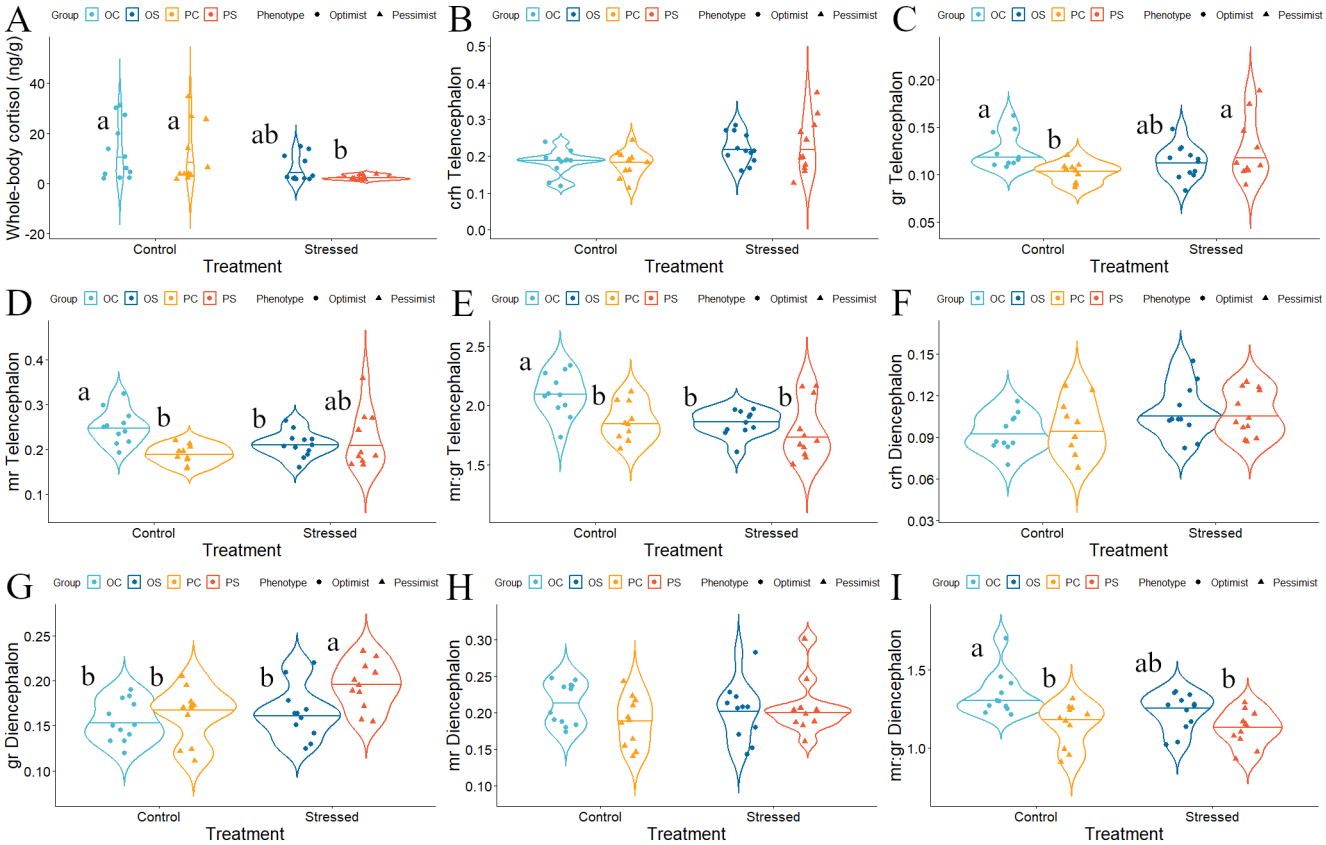
[a] Correlation between principal components and variable values
[b] Variance of transformed data used for each component

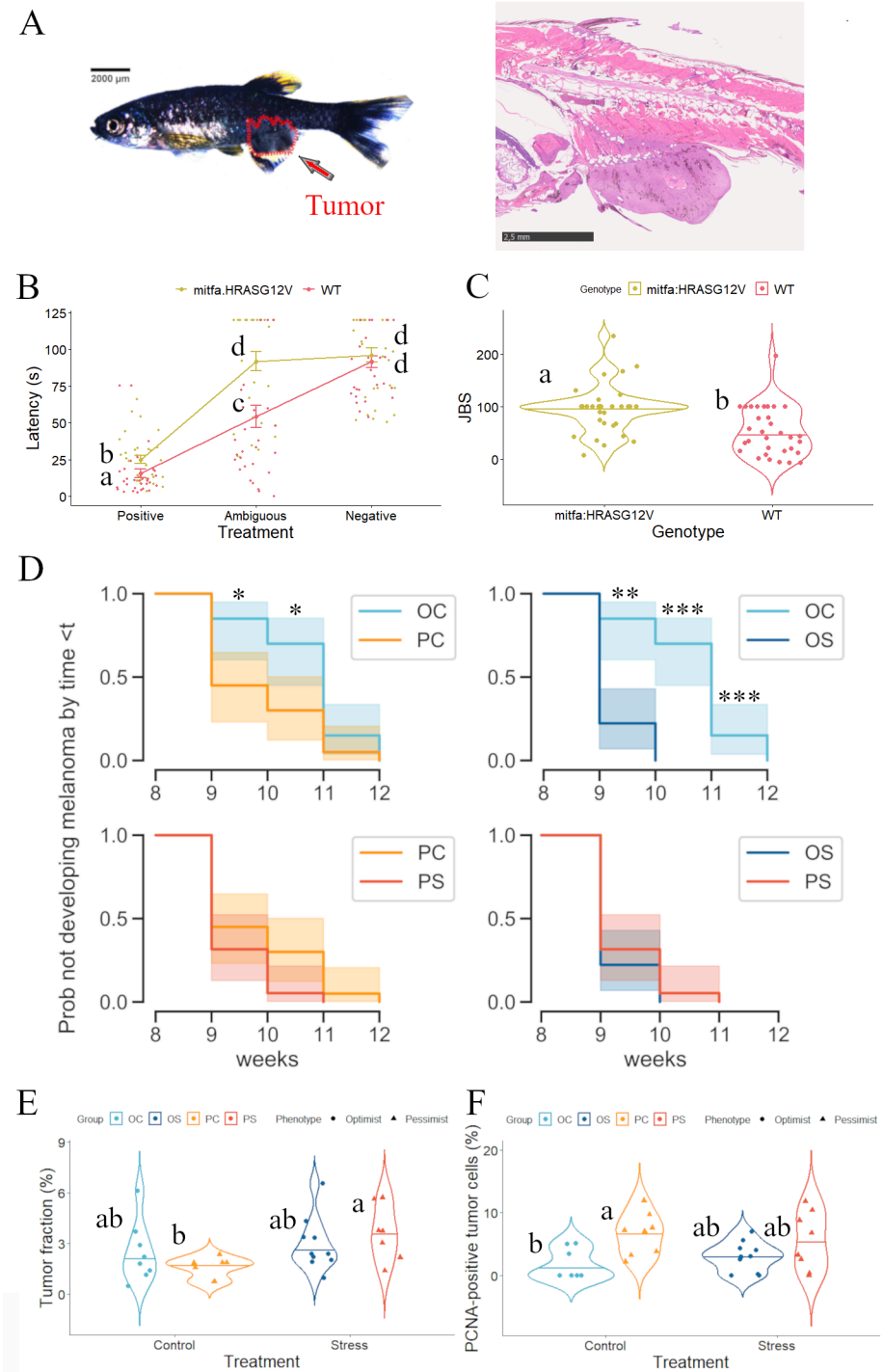
Tables

Table 1. **Principal component loadings from the correlation analysis**. Loadings extracted by principal component analysis from the correlation matrix of behaviors across tests for zebrafish belonging to the upper and lower quartiles of the JBS. Bold type indicates the strongest contributors (coefficient > 0.64) to each principal component (PC).


A
A
NP
NN
P
N
B
Density
0.02
0.01
0.00
0
30
60
90
Judgment Bias Score
C
Test 1
Test 2
Test 3
Latency (s)
60
40
20
0
Positive
Ambiguous
Negative
Treatment
D
Test
Test 1
Test 2
Test 3
JBS
100
0
Test 1
Test 2
Test 3
Test
E
Repeatability with CI
Frequency
Repeatability estimates
F
Repeatability with CI
Frequency
***
Repeatability estimates
G
H
I
OFT: Time in inner zone
OFT: Distance to wall
OFT: Number of transitions
OFT: Angular velocity
Exploration
PC1
NOT: Distance moved
OFT: Distance moved
Locomotor activity
PC3
NOT: Number of transitions
NOT: Time in novel object area
NOT: Angular velocity
NOT: Latency to first
JB: Judgement bias score
Bold-ness
PC2
1.0
0.8
0.6
0.4
0.2
0.0
J
PCA coordinates by phenotype
PC2 (25.2%): Boldness
PC1 (35.1%): Exploration
Phenotype:
Optimists
Pessimists


A
group
Control
Stressed
rps21
rps28
rpl39
rps29
RPL41
rpl29
atp5mea
atp5mf
atp5f1e
mtbl
ier2a
rnf213a
si:dkeyp-27c8.2
mid1ip1b
gadd45ba
BX323458.1
fads2
si:dkey-4p15.3
rtn4rl2a
egr4
btg2
fosab
npas4a
CR388166.1
zgc:122979
klf11a
prpf38b
pde6gb
rcvrn3
fbxl3l
avp
pdk2a
per1a
B
group
Control
Stressed
NCKAP1L
pgr
lpg
iqgap1
krt8
slc20a1b
cpxm1a
wu:fj16a03
kcnj2a
sulf1
itga8
slc38a4
bhmt
c4
clec11a
lamc3
cldn11a
fxyd1
apoeb
si:dkeyp-106c3.1
gpt2l
cp
slc22a7b.1
si:dkey-56d12.4
CABZ01074397.1
si:ch73-364h19.2
pltp
exd1
smox
glulb
opn1lw2
opn1lw1
pde6ha
grk1b
unc119.2
pdcb
rcvrn2
gngt2a
rd3
ENSDARG00000107716
si:ch1073-266p11.2
ntpcr
hoxa2b
pld6
nr1d4b
nr1d4a
spra
si:ch211-250k18.7
C
group
Control
Stressed
si:ch211-117k10.3
per1a
CABZ01074397.1
cdkn1d
pth2
tecpr2
kctd3
pycr1a
si:ch211-132g1.6
ENSDARG00000069159
ENSDARG00000034092
pmchl
prlh2
plxna1a
rbp4l
si:ch211-237l4.6
cldn19
zgc:92630
bcl6b
fa2h
plp1b
mpz
clk4a
BX936284.1
ppp1r14aa
zgc:63568
hsp90aa1.2
sptlc2b
mcm7
si:dkey-170l10.1
ENSDARG00000108666
prss35
msmo1
cyp51
SEPT4
tlcd1
hsd17b7
tpx2
eepd1
mid1ip1b
sqlea
klf11a
hells
si:dkey-56m19.5
sc5d
scarb2a
CR936442.1
tubb5
si:dkey-145p14.5
si:ch211-132b12.7
D
group
Control
Stressed
fbxl3l
ENSDARG00000075877
si:ch1073-266p11.2
nr1d4b
ENSDARG00000108952
CABZ01109058.1
pde6a
si:dkey-56d12.4
gpt2l
snu13a
E
PC vs PS - TEL
OC vs OS - DIEN
OC vs OS - TEL
PC vs PS - DIEN
43 (33%)
46 (35%)
1 (1%)
0 (0%)
0 (0%)
0 (0%)
0 (0%)
0 (0%)
29 (22%)
3 (2%)
0 (0%)
4 (3%)
5 (4%)
0 (0%)
0 (0%)
1 (1%)
count
40
30
20
10
0
F
Telencephalon
Diencephalon
Number of transcripts up- or down-regulated relative to each pairwise comparison
20
0
-20
-40
6
-27
18
-30
14
-36
7
-3
OC vs OS
PC vs PS
OC vs OS
PC vs PS
Pairwise comparisons
Genes
Downregulated
Upregulated


A
Group OC OS PC PS Phenotype Optimist Pessimist
Whole-body cortisol (ng/g)
a a ab b
Control Stressed
Treatment
B
crh Telencephalon
C
gr Telencephalon
D
mr Telencephalon
E
mr:gr Telencephalon
F
crh Diencephalon
G
gr Diencephalon
H
mr Diencephalon
I
mr:gr Diencephalon


A
2000 µm
Tumor
2.5 mm
B
mitfa.HRASG12V
WT
Latency (s)
Positive
Ambiguous
Negative
Treatment
C
Genotype
mitfa:HRASG12V
WT
JBS
mitfa:HRASG12V
WT
Genotype
D
Prob not developing melanoma by time <t
OC
PC
OS
PS
weeks
E
Group
OC
OS
PC
PS
Phenotype
Optimist
Pessimist
Tumor fraction (%)
Control
Stress
Treatment
F
PCNA-positive tumor cells (%)
Control
Stress
Treatment