Elevated glucocorticoid exposure during development alters behaviors and primes transcriptional responses to stress in adult zebrafish

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Abstract

Chronic or severe stress during development increases risk of adult diseases in humans. One putative mechanism involves priming of stress-sensitive gene networks by early life Glucocorticoids (GCs) exposure, which modifies responses to stress later in life. However, brain-wide GC-primed molecular alterations are poorly understood. Here, we used an optogenetic zebrafish model to increase endogenous GCs during development and identified comprehensive transcriptomic alterations in the whole brain of GC-exposed fish across the life course. When exposed to acute stress in adulthood, developmental GC-exposed animals show highly exaggerated and pervasive brain-wide transcriptional changes which include gene sets associated with axon development, neuronal signaling, and epigenetic modulators. Strikingly, these altered gene sets showed an overrepresentation of human psychiatric disorder risk genes. We identify in developmental GC-exposed individuals: 1) altered biological processes, 2) hitherto uncharacterized novel GC-primed genes, 3) altered adult behaviors, and 4) subsets of primed genes and putative transcriptional regulators associated with changes in adult social behavior including oxytocin and *myt1la*. Thus, our study establishes a new translationally relevant zebrafish model and provides molecular targets through which developmental GC-exposure may exert its life-long impact.

Significance statement

Chronic or severe stress during development has far-reaching consequences in shaping adult phenotypes and increasing psychiatric disease susceptibility in humans. Glucocorticoids (GCs) as conserved main stress hormones of vertebrates, play a pivotal in this process by "priming" certain genes for altered response in adulthood upon stress re-exposure. However, identities of GC-primed genes are only partially known. In our study, we examined brain-wide molecular consequences of developmental GC exposure using an optogenetic zebrafish model and identified altered biological processes, behaviors, and a comprehensive list of GC-primed genes. Interestingly, the GC-primed gene sets in zebrafish showed an overrepresentation of genes linked to human psychiatric disorders. We propose that GC-primed genes identified here may shape adult responses of early life stress-exposed individuals.

Introduction

Early life stress (ELS) can lead to long-term alterations in neural plasticity, functional connectivity, epigenetic dynamics, and behavior(1-4). Reflecting these far-reaching effects, ELS is a key factor affecting healthy development and aging as well as susceptibility to

psychiatric disorders in humans(5-8). The molecular response to stress in vertebrates is primarily mediated by a highly conserved neuroendocrine stress regulatory system called the Hypothalamo-Pituitary-Adrenal axis (HPA-axis) in humans(9) and the Hypothalamic-Pituitary-Interrenal axis (HPI-axis) in fish(10). Like the mammalian adrenal gland, the fish interrenal gland is composed of two cell types: aminergic chromaffin cells and steroidogenic interrenal cells, the latter of which produce glucocorticoids (GCs), effector stress hormones that are highly conserved across the vertebrate kingdom(9). While GCs have pleiotropic effects on many aspects of animal physiology, exposure to elevated GCs during brain development is known to cause long-term alteration of behavior, physiology, and stress regulation(1). However, how high GC exposure during development predisposes individuals to adulthood dysfunction is poorly understood. One potential mechanism is long-lasting epigenetic changes, which modify how animals respond to stress in adulthood. For example, altered DNA methylation at the glucocorticoid receptor (GR) gene promoter by poor maternal care is associated with adulthood alterations in histone acetylation, DNA methylation, transcription factor binding, GR expression, and HPA response to stress(11). Moreover, a recent study identified differentially expressed genes (DEGs) "primed" by ELS in specific regions of ELS-exposed mouse brains(12). These genes showed enhanced differential expression in adulthood upon re-exposure to stress and may be associated with behavioral changes in adulthood. Another study identified GC-primed molecular alterations using a GC (dexamethasone)-exposed human hippocampal progenitor cell line and identified long-lasting DNA methylation alterations(13). Lastly, genetic differences in the immediate transcriptome response to GR activation are shown to predict risk for depression and co-heritable psychiatric diseases(14). Together, the above studies establish that ELS or developmental GC exposure results in distinct responses to stress later in life by priming and maintaining longer-term alteration of specific genes which facilitate increased psychiatric disease susceptibility. However, so far, ELS or GC-primed genes have only been identified in specific regions of the brain including reward circuitry or cell lines, and brain-wide GC-primed molecular alterations have not been investigated.

In this study, we utilized optogenetic manipulation of steroidogenic interrenal cells in a zebrafish model to increase the endogenous GC level during larval and juvenile stages. Although the mammalian counterpart of the fish interrenal, the adrenal gland, is known to produce three types of steroid hormones, GCs, mineralocorticoids (MCs), and androgens(15), MCs and androgens are not detectable in larval zebrafish interrenal gland(16-19). Therefore, our manipulation is suitable to assess specific effects of elevating endogenous GCs. As a first step in determining the effects of developmental GC exposure, the zebrafish were exposed to

high levels of GCs throughout development starting from the 2 days post fertilization (dpf) representing an early postembryonic development stage.

Here we report brain-wide transcriptomic alteration of GC-exposed fish across the life course. Strikingly, when subjected to acute stress in adulthood, developmental GC-exposed fish show highly exaggerated endocrine and transcriptional responses, indicating that adult stress is processed differently depending on GC exposure history. Remarkably, the genes primed by GC-exposure were enriched in gene sets associated with human neuropsychiatric disorders indicating high levels of evolutionary conservation in how early GC exposure leads to adult dysfunction. We further identified 1) biological processes altered by developmental GC exposure, 2) adult behaviors altered by developmental GC exposure, 3) hitherto uncharacterized novel GC-primed genes, and 4) subsets of primed genes and putative transcriptional regulators associated with changes in adult social behavior induced by developmental GC exposure including oxytocin and *myt1la*.

Results

Optogenetic elevation of endogenous GC during development

We previously reported that elevation of endogenous cortisol (GC in fish) is induced in a transgenic zebrafish line by blue light present in ambient light which activates *beggiatoa* Photoactivated Adenylate Cyclase (bPAC), expressed specifically in steroidogenic interrenal cells(20, 21). For the present study, we generated a new transgenic line, *Tg(star:bPAC-2A-tdTomato)*^{uex300}, using the same construct in a different strain to obtain a higher cortisol level than what was previously feasible and raised the fish under ambient light (hitherto referred to as bPAC+) (Fig. 1a,b). Indeed, we observed a strong cortisol increase at 5 days post fertilization (dpf) in bPAC+ compared to bPAC-, siblings of bPAC+ which do not carry the transgene (Fig. 1c). bPAC+ larvae raised under a filter, which restricts blue light penetration, did not show a cortisol increase (Fig. 1c). Activation of the GR signaling cascade in bPAC+ was confirmed through increase in the level of *fkbp5*, which regulates the responsiveness of the glucocorticoid receptor (GR)(22) (Fig. 1d). Since transgenerational inheritance of stress-induced phenotypes is reported in mammals and fish(23-25), we compared bPAC+ and bPAC- to each other, as well as to wild type in this study. Consistent with the reported onset of endogenous *star* gene expression(26), an increased cortisol level was observed starting around 2 dpf (Fig. 1e). The high cortisol level was sustained during larval and juvenile stages in bPAC+ fish. However, by adulthood (three months post-fertilization (mpf)), no significant difference was detected between bPAC+ and wild type (Figs. 1e,f). This may be due to the decrease in blue-light

124 penetration following the reduced transparency of the skin as fish grows. These results indicate
125 that by using bPAC+ we can achieve a robust and persistent increase in endogenous cortisol
126 levels throughout development.
127
128 **Alteration of adult behavior in developmental GC-exposed fish**
129 We assessed whether bPAC+ fish exhibited behavioral changes as adults using a set of tests
130 aimed at assessing behaviors that can be affected in neuropsychiatric diseases(27). We first
131 established that bPAC+ fish did not show significant differences in basal locomotor activity
132 compared to wild-type and bPAC- fish (Figs. 2a,b). We then focused on social behavior as
133 social processes are translatable across multiple species and are often perturbed in a range of
134 disorders associated with ELS(28). To assess social behavior, we employed a new test that we
135 recently established, which allows the assessment of distinct phases of social behavior, namely
136 a drive to approach a social cue, and an ability to engage and maintain social interaction (Fig.
137 2c)(29). In this test, after the fish was placed in a test tank and before the presentation of
138 conspecifics, wild type, bPAC- and bPAC+ all displayed preference for the blue-colored
139 bottom zone with no significant genotype differences (Figs. 2d,e). When the social cue was
140 revealed, within 10 seconds the vast majority of wild type, bPAC-, and bPAC+ entered the
141 social interaction zone (shaded green in Fig. 2f) adjacent to the conspecifics, within which wild
142 types display a stereotypical C-shaped pattern of swimming (Fig. 2h). No difference in social
143 approach between the groups was found using a Log-rank test (P=0.77), indicating that bPAC+
144 did not show deficits in recognition nor approach to social cues (Fig. 2g). In contrast, we found
145 significant differences between wild type and bPAC+ during the 2-minute social phase in the
146 time spent in the interaction zone and the number of social interactions performed (Figs. 2h,j).
147 bPAC- also spent less time in the interaction zone and performed fewer interactions than the
148 wild type (Figs. 2i,j).
149 Next, we tested food consummatory behavior by counting the number of floating food pellets
150 eaten by a fish during a 10-minute interval (Supplementary Fig. 1a). Wild-type adult fish
151 consumed on average 14.5 pellets while bPAC+ fish consumed on average 2.33 pellets out of
152 a total of 25 (Supplementary Figs. 1b,c). This was not due to the lack of recognition nor interest
153 in the food, as bPAC+ fish approached the food pellets and actually spent a longer time in the
154 zone near the pellets (feeding zone) than wild types (Supplementary Fig. 1d).
155 To assess cognitive processes, we tested associative learning using a Pavlovian avoidance
156 learning test where fish learn to associate the color green with a mild electric shock in the
157 testing chamber (Supplementary Figs. 1e,f). Fish were first habituated in the chamber for 30

158 minutes and tracked to determine the baseline preference for green or blue color. Wild-type
159 and bPAC+ fish showed no significant differences in baseline color preference (Supplementary
160 Fig. 1g). However, after the conditioning, wild-type fish spent on average 89.2% of their time
161 in the blue zone, significantly more than bPAC+ fish who spent on average 62.68% of their
162 time in the blue zone (Supplementary Fig. 1h), suggesting a deficit in associative learning in
163 bPAC+ fish.
164 Lastly, we tested the behavior of bPAC+ fish in a novel environment using a novel tank test
165 (Supplementary Figs. 1i-m). In the novel tank test, wild-type fish first dive to the bottom and
166 demonstrate a sustained depth-preference whilst performing bouts of erratic high-speed
167 swimming(30, 31). In contrast, bPAC+ fish showed reduced depth-preference (Supplementary
168 Fig. 1j) and generally swam slower than wild-type controls (Supplementary Fig. 1k). Time
169 spent performing fast-swimming bouts was also significantly decreased in bPAC+ fish
170 (Supplementary Fig. 1l). However, there was no significant difference in freezing duration
171 between groups (Supplementary Fig. 1m). These results suggest altered novelty perception and
172 processing in bPAC+ fish.
173 Together these results show that social interaction, food consumption, associative learning, and
174 adaptation to a novel environment are altered in bPAC+ fish compared to wild types. Our
175 results point to modification of a broad range of goal-directed behavior performance without
176 changes in basal locomotion parameters in bPAC+.
177
178 **Whole-brain transcriptional alterations in bPAC+ fish across the life course**
179 Whole-brain RNA sequencing was carried out using larval (6 and 13 dpf) and adult (120 dpf)
180 brains to identify and compare transcriptomic differences in wild type, bPAC+, and bPAC-. A
181 large number of DEGs was identified in pairwise comparisons of bPAC+ and wild-type brains
182 at 6 dpf (up/down=602/1253), 13 dpf (up/down=777/1566), and 120 dpf (up/down=684/1078)
183 (Fig. 3, Supplementary Table 1). At all-time points, the number of downregulated genes was
184 greater than the number of upregulated genes, indicating that developmental GC-exposure
185 tends to repress rather than activate genes (Fig. 3a). Further, the number of DEGs consistently
186 downregulated from early life until adulthood (273 DEGs) was greater than the upregulated
187 DEGs (48 DEGs) (Fig. 3a). We identified fewer DEGs when comparing bPAC- to wild type,
188 with 571 (up/down=329/242), 1026 (up/down=372/654), and 74 (up/down=42/32) DEGs at 6,
189 13, and 120 dpf, respectively, and many of these DEGs overlapped with those identified in the
190 bPAC+/wild type comparison (Supplementary Fig. 2a). To compare the overall transcriptomic
191 landscape of all identified DEGs, we performed unsupervised hierarchical clustering, which

192 revealed large differences in the gene expression landscape between the bPAC+ and bPAC-
193 compared to wild type (Fig. 3b). At each time point, bPAC+ and bPAC- transcriptome profiles
194 clustered together separately from those of wild type. Next, to confirm an increase in GR
195 signaling, we measured the expression level of GR target genes, *nr3c1, nr3c2*, *fkbp5*, *tsc22d3,*
196 and *hsd11b2* at 6, 13, and 120 dpf. The expression levels of *nr3c1, fkbp5, tsc22d3,* and *hsd11b2*
197 were altered at multiple time points in bPAC+ (Fig. 3c). bPAC- also showed differences to
198 wild type, albeit generally to a lesser degree (Fig. 3c). Both the alteration of GR signaling and
199 similarity of DEGs identified in bPAC- and bPAC+ point to potential transgenerational effects
200 present in bPAC- resulting from high GC-exposure in the parental generation. Supporting this
201 notion, even though bPAC- was not exposed to a high level of GC, some of the DEGs at 6 dpf
202 in bPAC- are involved in processes that potentially regulate GC inactivation (Supplementary
203 Fig. 2b, Supplementary Table 2).
204
205 **Developmental GC exposure leads to downregulation of transcripts involved in nervous**
206 **system development**
207 To identify molecular consequences of developmental GC exposure, we characterized DEGs
208 in bPAC+ compared to the wild type. Upregulated DEGs in bPAC+ fish brains at 6 dpf were
209 enriched in the GO terms that include potential regulatory enzymes for GC such as cytochrome
210 monooxygenases, 11β-hydroxysteroid dehydrogenase or sulfotransferases(32-34) (left panel
211 of Fig. 3d). At 13 dpf, the top-ranked enriched biological processes for upregulated DEGs were
212 cell cycle, chromosome segregation, and DNA metabolic process which can cause genomic
213 instability by replication stress(35) (left panel of Fig. 3d). At 120 dpf, bPAC+ brains showed
214 upregulation of genes involved in ribonucleoprotein complex biogenesis, peptide metabolic,
215 translation, protein modification, RNA processing and more, suggesting effects of
216 developmental GC exposure on gene expression regulation and post-transcriptional
217 modification in adulthood (left panel of Fig. 3d, Supplementary Table 2). In contrast,
218 downregulated DEGs across all time points were mainly enriched in processes related to cell
219 adhesion and cell migration, as well as nervous system development including neuron
220 differentiation, cell morphogenesis, and axon development (right panel of Fig. 3d,
221 Supplementary Table 2). Brain-wide analyses using qPCR and immunohistochemistry
222 confirmed alteration of neurogenesis in larval bPAC+ fish. These results are reported in detail
223 in a separate report(36). During larval stages, downregulated DEGs were also enriched in
224 anatomical structure formation, muscle structure development, vasculature development,
225 external encapsulating structure organization, and more. We also identified functions

226 associated with time-point-specific downregulated DEGs (Supplementary Table 2). For
227 example, DEGs at 6 dpf were enriched in brain development while DEGs at 13 dpf were
228 enriched in the cellular response to organic substance (right panel of Fig. 3d). At 120 dpf, we
229 identified downregulated DEG enrichments in synaptic signaling, regulation of molecular
230 function, and regulation of GTPase activity and more (right panel of Fig. 3d).
231 These results highlight long-lasting downregulation of neuronal development and signaling
232 processes, and adult-onset alteration of transcription and translation regulation following
233 developmental GC exposure.
234
235 **bPAC+ fish show an enhanced endocrine response to acute stress in adulthood**
236 To assess whether high GC exposure during development affects the stress response in
237 adulthood, we subjected wild-type, bPAC+, and bPAC- fish to a looming dot stimulus (LD),
238 which mimics an approaching predator(29) (Figs. 4a,b). Interestingly, bPAC+ adults exhibited
239 a much greater cortisol increase after LD exposure compared to wild-type adults (Fig. 4c),
240 indicative of a sensitized endocrine response to acute stress in bPAC+. Similarly, we found an
241 altered transcriptional response of stress-associated genes in bPAC+ after LD exposure (Fig.
242 4d). For example, the expression of *nr3c2*, the gene coding for the mineralocorticoid receptor
243 (MR), was further downregulated only in bPAC+ adults following LD exposure, although basal
244 expression of *nr3c2* was already decreased in bPAC+ compared to wild type. In contrast, the
245 GC-inactivating enzyme coding gene, *hsd11b2,* was significantly upregulated in the wild type
246 after LD exposure, but upregulated to a lesser degree in bPAC+ following LD exposure. Given
247 the higher affinity of MR to GC, MR can be a regulator of GR signaling in MR and GR co-
248 expressing cells in basal conditions(37, 38). Moreover, reduced transcriptional reactivity of
249 *hsd11b2,* a key enzyme for cortisol inactivation(39, 40), in bPAC+ after LD may lead to an
250 increased accumulation of active cellular cortisol. These results suggest that developmental
251 GC-exposure may lead to a sensitized response to stress by decreasing MR expression at pre-
252 and post-LD and attenuating the increase of *hsd11b2* expression.
253
254 **Developmental GC exposure primes large-scale differential gene expression following**
255 **acute stress in adulthood**
256 To identify the transcriptional response to acute stress following developmental GC exposure,
257 we carried out whole-brain RNA sequencing of wild type, bPAC+ and bPAC- that had been
258 exposed to LD in adulthood and made three separate comparisons (Figs. 4e,f). First, we
259 compared the wild-type transcriptome at basal (pre-LD) and after LD exposure (post-LD), to

identify the normal acute stress response in wild-type fish (referred to as DEG1). Secondly, we compared pre-LD and post-LD in bPAC+ (referred to as DEG2), and finally the post-LD transcriptome of wild type and bPAC+ was also compared (referred to as DEG3). DEG3 includes LD-induced DEGs (overlapping with DEG1 and DEG2) as well as those DEGs that were already altered in bPAC+ prior to LD exposure and therefore the number of DEG3 genes (2034 genes) was greater than that of DEG1 and DEG2 (Fig. 4g). Strikingly, bPAC+ showed more than 20-fold greater number of DEGs in response to LD exposure (DEG2, 1617 genes) compared to wild type (DEG1, 64 genes) (Fig. 4g). Altogether we identified 2515 genes that appeared to be altered in the adult brain by developmental GC exposure in bPAC+, and refer to them collectively as Adult bPAC+ DEGs.

The exaggerated transcriptional LD response in bPAC+ indicates that developmental GC exposure in zebrafish primes enhanced alteration of gene expression in adulthood, similar to observations in previous mammalian ELS or GC-primed gene studies(12, 13). To define GC-primed genes, we took the entire DEG2 set and excluded 44 genes that overlapped with DEG1 as these represent normal acute stress-induced genes present also in the wild type. To this gene set, we combined the 18 genes in DEG1 that do not overlap with DEG2 as these genes are reflective of wild-type responses that are missing or altered in bPAC+ (Fig. 4g, Supplementary Table 3). This set of 1591 genes is collectively referred to as GC-primed genes here and represents those genes that are primed by developmental GC exposure to produce an altered transcriptional response to acute stress in adulthood. Among the 1591 GC-primed genes, 1337 downregulated genes were enriched in neuron projection development, synaptic signaling, receptor complex, synapse, metal ion transmembrane transport, and more (Fig. 4h, Supplementary Table 4).

To compare GC-primed genes in zebrafish with those identified in humans, we carried out a comparison with a recent study that reported 702 GC-primed DEGs linked with long-lasting differentially methylated sites following re-exposure to GC (dexamethasone)(13). These DEGs were identified in GC-treated mature neurons which were exposed to GC during neurogenesis in a human hippocampal progenitor cell (hpc) line. Through the comparison between 702 DEGs (hpc_ELS-primed genes, up:368, down:334)(13) and our DEG3, which represents a similar GC re-exposed condition, we found 163 overlapping genes (up/down=99/64) (Supplementary Fig 3, Supplementary Table 5).

Together, these results reveal for the first time a brain-wide set of genes primed by developmental GC exposure. Further, we found a significant overlap between subsets of GC-primed genes identified in zebrafish with those from mammalian studies, suggesting a high

294 degree of evolutionary conservation of molecular mechanisms underlying reprogramming by
295 GC.
296
297 **Adult bPAC+ DEGs are associated with neuropsychiatric disorders in humans**
298 Since stress exposure during development is a risk factor for the development of psychiatric
299 disorders in humans(6-8), GC-primed genes were compared with known psychiatric disease
300 risk factors using the curated DisGeNET database(41). Strikingly, human homologs of GC-
301 primed genes exhibit a significant enrichment of known psychiatric disease-associated genes
302 (Supplementary Table 6). Top-ranked psychiatric disorders were schizophrenia, bipolar
303 disorder, and autistic disorder (Supplementary Fig. 4). Further, the entire adult bPAC+ DEG
304 set was strongly enriched in known psychiatric disease risk factors and in this case depressive
305 disorder was also included in the top four ranked diseases (Figs. 5a,b, Supplementary Table 7).
306 Upon LD exposure, the difference in expression of schizophrenia-associated genes in bPAC+
307 was greatly enhanced, as represented by the size of nodes in Fig. 5c. Further, schizophrenia-
308 associated Adult bPAC+ DEGs were enriched in either of two KEGG pathways: neuroactive
309 ligand-receptor interaction (FDR=2.01e-08), or MAPK signaling pathway (FDR=9.74e-08).
310 Overall, across all four disease categories, the top-ranked disease-associated Adult bPAC+
311 DEGs were mainly enriched in the downregulation of synaptic signaling, and multicellular
312 organismal process (Fig. 5d).
313 Adult bPAC+ DEGs associated with schizophrenia, bipolar disorder, depressive disorders, and
314 autistic disorders were predominantly downregulated following LD exposure (Fig. 5e).
315 Interestingly, we noted that some of the disease-associated Adult bPAC+ DEGs were already
316 differentially expressed from the larval stage (trans-temporal, 60 genes) while some others
317 showed alteration in their expression only upon exposure to acute stress in adulthood (adult-
318 onset, 65 genes) (Fig. 5f). Other non-disease-associated Adult bPAC+ DEGs maintained
319 differential expression from the larval stage (540 genes) or exhibited an enhanced alteration
320 upon adulthood stress (adult-onset, 463 genes) (Supplementary Table 8). Trans-temporal Adult
321 bPAC+ DEGs were mostly enriched in axon development (FDR=1.33e-08, 31 DEGs), whilst
322 adult-onset Adult bPAC+ DEGs were enriched in ion transport (FDR=7.26eE-05, 44 DEGs)
323 (Supplementary Table 9). Interestingly, a cell-type enrichment test using the human homologs
324 of Adult bPAC+ DEGs showed that 24 trans-temporal Adult bPAC+ DEGs were associated
325 with non-microglial oligodendrocyte lineages. In contrast, the downregulated adult-onset adult
326 bPAC+ DEG set was associated with non-microglial neuron-enriched genes including
327 GABAergic receptor-associated genes (*GABRA1*, *GABRG2*), other ion channel activity genes

328 (*SLC12A5*, *CACNA1E*, *CACNA1I*, *UNC80*, *KCNH1*, *SCN2B*), and genes associated with
329 synapse and neuronal cell signaling (*DNM1*, *DLGAP2*, *RPH3A*, *GPR158*) (Supplementary
330 Table 10). More than half of those genes were GC-primed genes. These results suggest that
331 developmental GC-exposure has cell-type-specific impacts.
332
333 **Altered gene expression associated with epigenetic modifications and RNA processing in**
334 **bPAC+ fish**
335 To identify underlying mechanisms by which developmental GC exposure regulates gene
336 expression in the brain, we analyzed the expression of epigenetic modifiers. We identified 70
337 Adult bPAC+ DEGs associated with epigenetic modifications (Supplementary Figs. 5a,b,
338 Supplementary Table 11). These genes are associated with RNA modification (up/down=3/3),
339 RNA processing including mRNA splicing (up/down=27/17), histone modification
340 (up/down=4/18), and DNA modification (up/down=0/3). Among them, the DNA
341 methyltransferase (DNMT) 3aa gene and the histone modification-associated genes, *chd3*,
342 *hdac4*, *hdac5*, *kdm2ba*, *kdm4aa*, *mta3*, *nsd3*, *padi2*, *suv39h1b*, and *yeats2,* showed consistent
343 downregulation across time-points.
344 We also found significant differences in the expression of 4 out of 7 *DNMTs* in the zebrafish
345 genome including *dnmt1*, *dnmt3aa*, *dnmt3ab,* and *dnmt3bb.3* across time points and conditions
346 in bPAC+ compared to the wild type (Supplementary Fig. 5c). In the wild type, *dnmt1* is highly
347 expressed at 6 dpf and expression then gradually decreases over time, but in bPAC+ expression
348 was significantly reduced at 6 dpf. Expression patterns of *dnmt3aa* and *dnmt3ab*, orthologous
349 to human *DNMT3A,* known as one of the writers of *de novo* methylation, mainly showed
350 differences in bPAC+ adult brains. Further, low expression of *dnmt3aa* and upregulation of
351 *dnmt3ab* was observed upon LD exposure in adulthood in bPAC+. To determine whether
352 alteration of the most differentially regulated epigenetic modulators (labeled with * in
353 Supplementary Fig. 5a) lasts throughout adulthood, we analyzed their expression at 8 mpf, with
354 and without LD exposure. Downregulated expression of those genes was continuously
355 observed in the later adult stage (8 mpf) mainly upon acute stress in bPAC+ adults
356 (Supplementary Fig. 5d). These results suggest that developmental GC exposure leads to
357 pervasive, long-lasting changes to epigenetic modulators.
358
359 **Social behavior-associated genes are a part of the GC-primed genes**
360 To search for a potential link between the observed molecular changes and social behavior, we
361 analyzed the expression patterns of 101 known social behavior-associated genes from the Gene

362 Ontology Annotation database (henceforth referred to as social genes). Interestingly, 54 of
363 these social genes were differentially expressed in bPAC+ brains, where 44 were
364 downregulated at least at a single time point (|FC|>1.5 and FDR<0.05) and only 11 were
365 upregulated (*npas4a* was double counted because downregulated at 6 dpf and upregulated at
366 13 dpf, Fig. 6a, upper panel). In the basal condition, 6 dpf brains showed the largest change
367 (up/down=2/31). The most significant downregulated social genes over time were *nlgn2b*,
368 *mapk8ip2*, *brinp1*, *slc6a4a,* as well as oxytocin (*oxt*), a well-known social gene(42) (Fig. 6a).
369 bPAC- showed a similar trend in the downregulated gene expression pattern following LD
370 exposure as in bPAC+, albeit the level of difference was lower though. (Fig. 6a, bottom panel).
371 We confirmed the downregulation of *oxt* both in larval and adult bPAC+ brains using qPCR
372 (Fig. 6b). Downregulated *oxt* expression and the number of oxt+ cells in larval brains were also
373 confirmed using immunohistochemistry which showed a preferential reduction of the posterior
374 cluster of oxt+ cells which is likely to be similar to mammalian parvocellular oxt+ neurons
375 based on their projection(43, 44) (Figs. 6c,d).
376 Notably, *oxt* regulators including *myt1la*, *med12*, and *thrab* were downregulated at 6 dpf and
377 two of them (*myt1la*, *med12*) remained downregulated until adulthood (Fig. 6e). Further,
378 transcription factor (TF) enrichment analysis of human orthologues of the downregulated
379 social genes identified *myt1la* as one of the top regulators of 47 differentially expressed social
380 genes, based on the ChEA3 database(45) (Fig. 6f). These results suggest that downregulation
381 of *myt1la* in bPAC+ may result in the altered expression of social genes including *oxt*(46).
382 Strikingly, a subset of social genes was also part of the GC-primed genes indicating that the
383 regulation of their adult expression is highly sensitive to the history of developmental GC
384 exposure (red label in Fig. 6a).
385 Beyond social genes, we identified common potential upstream regulators of GC-primed genes
386 using the ChEA3 platform(45) (Supplementary Figs. 6a,b, Supplementary Table 12). For the
387 upregulated GC-primed genes, immediate early genes were top-ranked TFs. Among the top-
388 ranked TFs for downregulated GC-primed genes, *camta2* and *myt1l* may be involved in the
389 regulatory process of 149 and 139 downregulated genes, respectively. Expression of *myt1l* was
390 consistently downregulated in bPAC+ adults at both pre-LD and post-LD conditions
391 (Supplementary Fig. 6c). Together, these results identify potential developmental GC-induced
392 regulators of adult social behavior.
393
394 **Discussion**

395 This study examines the effects of chronic developmental GC exposure in shaping adult
396 phenotypes at both behavioral and molecular levels. We report altered goal-directed behaviors
397 in adults and molecular consequences of GC-exposure in both larvae and adults. Further, we
398 report a comprehensive identification of genes differentially expressed in adulthood following
399 developmental GC exposure, many of which correspond to genes associated with
400 neuropsychiatric disorders in humans. These findings suggest that developmental GC exposure
401 primes differences in acute stress-induced transcriptional activation in adulthood and may
402 increase the risk for neuropsychiatric disorders by affecting a network of stress-sensitive genes.
403
404 We identified brain-wide alterations in bPAC+ through whole-brain transcriptome analyses
405 using both larval and adult zebrafish. Downregulated genes in bPAC+ are mainly enriched in
406 cell morphogenesis, neuronal cellular signaling, and neuropsychiatric disorders-associated
407 gene sets. Despite the differences in species, stressor type, and brain region, the results of our
408 GO enrichment test and observed phenotypes are broadly similar to previous findings obtained
409 from ELS rodent models which report alterations in axon development, synaptic density and
410 plasticity, altered hippocampal mossy fibres(4, 5, 47). However, we identified a greater number
411 of DEGs when compared to previously reported mammalian ELS paradigms consistent with
412 the fact that the model described here represents a stronger and longer stress exposure.
413 Interestingly, we identified a large set of adult-onset upregulated DEGs which were enriched
414 in DNA metabolism, various RNA processing processes, and posttranslational modifications.
415 This may indicate that developmental GC-exposure can lead to genome instability by DNA
416 replication stress and altered gene expression regulation via alternative splicing, non-coding
417 RNAs, and posttranslational modifications in adulthood.
418
419 Identified Adult bPAC+ DEGs were enriched in neuropsychiatric disorders when compared to
420 a publicly available catalogue of disease-associated genes based on gene expression network,
421 eQTLs and GWAS(41). The majority of schizophrenia, bipolar disorder, depressive disorder,
422 and autistic disorder-associated Adult bPAC+ DEGs showed an exaggerated transcriptional
423 response to stress in adult bPAC+ brains. Those DEGs were overrepresented in synaptic
424 signaling processes including genes for GABA receptors (*gabrg2*, *gabbr1a*, *gabbr1b*, *gabbr2*,
425 *gabra1*, *gabra2a*, *gabra4*, *gabra5*, *gabrg1*, *gabrg2*), glutamate receptors and transporter
426 (*grm1b*, *grm3*, grm5b, *slc17a7a*), dopamine receptors and transporter (*drd1b*, *drd2a*, *drd2b*,
427 *slc6a3*), serotonin receptor (*htr1b*), cholinergic receptor (*chrm2a*), opioid receptor (*pnoca*), and
428 neurexins (*nlgn1*, *nlgn2b*, *nlgn4xb*). In a human study, genetic variants associated with GR-

429 mediated immediate transcript response were able to predict risk for psychiatric disorders
430 including depression(14). Consistent with this, our findings suggest developmental GC
431 exposure leads to differences in GR-induced transcriptional activation in adult brains and may
432 mediate the risk for psychiatric disorders by altering a network of neuronal signaling-related
433 stress-sensitive genes.
434
435 Growing evidence indicates that GC signaling can lead to modifications to DNA methylation,
436 histone codes, and non-coding RNAs(48-50). Similarly, we identified significantly altered
437 expression of epigenetic modulators. One of the most significantly altered epigenetic regulators
438 in bPAC+ brains are *DNMTs* (*dnmt1* and *dnmt3aa*) at the larval stage (6 dpf), which may result
439 in a global or local reduction in DNA methylation. DNMT1 mainly plays a role in the
440 maintenance of DNA methylation(51) while accumulation or gain of DNA methylation is
441 achieved by DNMT3a and -3b(52). Given such far-reaching effects of each DNMT, alteration
442 of expression levels of dnmt genes that we observe in bPAC+ is predicted to change the global
443 DNA methylation landscape across the life course. In our study, we identified consistently
444 downregulated Adult bPAC+ DEGs associated with histone modifications across the life
445 course. Temporal regulation of chromatin structure via the "histone code" in neurons has been
446 regarded as a crucial factor for dynamic plasticity in the mature nervous system and long-
447 lasting neuronal responses(53). Among the histone modulator genes, *hdac4* and *hdac5* were
448 consistently downregulated in bPAC+ fish, and have been implicated in depression, fear
449 learning, PTSD, eating disorder (Anorexia nervosa), and schizophrenia(54-59). Furthermore,
450 many epigenetic-associated Adult bPAC+ DEGs are involved in RNA processing and RNA
451 editing. Among them, we found a downregulation of *adarb2* and *pum2* in bPAC+ fish after
452 exposure to LD. Chen et al. suggest that ADARB2 has a regulatory role in RNA editing by
453 inhibiting the binding of other adenosine deaminases acting on RNA (ADARs)(60) and
454 ADARs are associated with the development of epileptic seizures in mice and depressive
455 phenotypes(61, 62). Further, *pum2* is involved in axon growth and branching(63). However,
456 further investigation is required to reveal direct mechanisms between the identified Adult
457 bPAC+ DEGs and alterations of epigenetic modulators.
458
459 As early exposure to excess GC is considered a global risk factor for the development of mental
460 disorders, our behavioral pipeline was tailored to assess performance in key behavioral
461 manifestations rather than disease-specific tasks. bPAC+ fish did not show defects in aspects
462 of perception as they can recognize visual and olfactory stimuli in the form of social cues and

463 food pellets. However, bPAC+ fish showed deficits in fear learning using a pavlovian
464 conditioning paradigm that pairs electric shock with the color green. It is possible that
465 perceived salience of electric shock as a stressor rather than fear learning is diminished in
466 bPAC+. However, we consider this possibility less likely as bPAC+ can respond robustly to
467 acute stress presented in the form of LD. This phenotype is consistent with what has been
468 reported in rodent ELS models, which showed deficits in fear learning, learning impairment,
469 and decreased cognitive flexibility(64-66). In zebrafish, fear-conditioned avoidance learning is
470 a robust phenotype whereby fish exhibit freezing and erratic behavior upon re-exposure to the
471 conditioned stimulus (CS)(67, 68). Although one study found that chronic unpredictable stress
472 did not have any effect on fear conditioning in adult zebrafish(69), a different ELS protocol
473 found improvement of short-term memory by early life stress when testing in the unconditioned
474 y-maze(70). Differences in how zebrafish learn fear association are dependent on whether they
475 are reactive or proactive responders to stress, raising the question of how bPAC+ fish's
476 response fits into that spectrum(71).
477
478 bPAC+ fish exhibited a reduction in the maintenance of social interactions. Similarly in
479 previous zebrafish ELS studies, diminished shoaling behavior was observed following early-
480 life social isolation and was associated with both motivational deficiencies linked to
481 dopaminergic systems(72) and increased sensitivity to stimuli which was reversed by
482 anxiolytic treatment(73). Impairments in social behavior or social withdrawal are common
483 among different psychiatric disorders, including autism(74) and schizophrenia(75), and have
484 been described in rodent models of ELS in which deficits in social interaction in the absence
485 of social recognition and approach have been observed(76). Interestingly, neonatal handling
486 and short bouts of maternal separation in male rats resulted in reduced oxytocin neurons in the
487 parvocellular region of the paraventricular nucleus (PVN) of the hypothalamus and a reduction
488 in the time spent interacting and sniffing, with no differences in recognition or approach
489 reported(76). We observed reduced expression of oxytocin, in bPAC+ brains during the larval
490 stage, and this downregulation persisted until adulthood, although the extent of the difference
491 decreased over time. Consistent with our observation that posterior oxt+ cluster is selectively
492 reduced in bPAC+, a subset of parvocellular oxt neurons enriched genes identified in recent
493 scRNA-seq results of mice were downregulated in bPAC+ (such as *brinp1*, *baiap3*, *pacsin1a*,
494 *syt17*, and *rcan2*)(77). This may suggest that subgroups of oxt+ neurons are differentially

495 controlled by ELS during development and play distinct roles in ELS-induced modification of
496 stress responses and social behavior.
497
498 In conclusion, we show that developmental GC exposure leads to altered behaviors and brain-
499 wide transcriptional changes that were enriched in the processes of DNA metabolism, nervous
500 system development and neuronal signaling across the life course. We identified brain-wide
501 Adult bPAC+ DEGs that are overrepresented in gene sets associated with neuropsychiatric
502 disorders and are enriched in epigenetic modulators. Further, developmental GC exposure led
503 to exaggerated endocrine responses and primed a set of genes to exhibit altered transcriptomic
504 responses to subsequent challenges in adult fish. Lastly, we identified reduced expression of
505 *oxt* and its regulators such as *mytl1a* in bPAC+ which may contribute to the altered social
506 behavior phenotype in bPAC+. We propose that the brain-wide DEG set identified here will
507 be useful for guiding future studies dissecting the mechanism underlying developmental GC-
508 exposure and behavioral dysfunction and neuropsychiatric disease susceptibility in adulthood.
509 The zebrafish model presented here provides an opportunity to dissect the long-term effects of
510 endogenous GC elevation during development and can be leveraged in future studies to identify
511 critical period, intensity, and duration of developmental GC exposure that leads to adult
512 dysfunction.
513
514 **Methods and materials**
515 The detailed protocol for all the procedures used in this study is available as step-wise online
516 methods(78).
517
518 **Zebrafish husbandry and maintenance**
519 The tübingen (TU) strain was kept at 28°C on a 12:12 hour light/dark cycle and housed at a
520 maximum density of 5 fish/L. For experiments with adult fish, only females were used. All
521 animal procedures were carried out in compliance with the ethical guidelines of the national
522 animal welfare law and approved by relevant authorities (Landesuntersuchungsamt
523 Rheinland-Pfalz, Germany, Project number 23 177-07/G20-1-033 or UK Home Office PPL
524 number PEF291C4D).
525
526 **Generation of transgenic line**
527 Linearized pT2-2kbStAR:bPAC-2A-tdTomato(20) (ZFIN ID: ZDB-GENE-001120-3)
528 recombinant plasmids were injected into 1-cell stage wild-type embryos of TU strain at 10-50

529 ng/mL in the presence of 10-50 ng/mL Tol2 transpose mRNA and 0.05% phenol red. The
530 progenies of injected fish were screened for interrenal-specific tdTomato expression and the
531 stable transgenic line, *Tg(star:bPAC-2A-tdTomato)*^{uex300} was established.
532
533 **Sample collection**
534 All samples were collected within a 2-hour window in the morning (08:30 to 10:30) without
535 feeding.
536
537 **Whole-body Cortisol assay**
538 The competitive cortisol assay (Cisbio HTRF® Cortisol Kit, 62CRTPEG) was performed
539 following the manufacturer's protocol. ELISA signal was detected by CLARIO star plate
540 reader (BMG Labtech, Ortenberg, Germany).
541
542 **Behavior tests**
543 Social behavior tests and acute stressor delivery using looming dot (LD) presentation were
544 carried out as described in Cook *et al.*(29). Basal locomotion, food consumption and fear
545 conditioning assay were performed following the protocol in online methods(78). For all
546 behavioral tests, female adult zebrafish (6-9 months old) were moved into a designated
547 behavior experimental room one week prior to testing and housed in groups of 10 fish in 3L
548 tanks. Experiments were carried out between 09:00 and 14:00 and fish were not fed in the
549 morning of testing. Fish were not used in any repeat experiments. The trials were recorded at
550 30 FPS. A camera (acA1300-60gm, Basler ace) was positioned at the front and/or top side of
551 the tank. The test fish was tracked using Ethovision (EthoVision XT 13, Noldus Information
552 Technology).
553
554 **RNA preparation and quantification**
555 Each sample for the larval stage (6 and 13 dpf) contained 25-30 larval whole brains whilst for
556 the adult stage (120 dpf) three brains constituted one sample. Transcripts were quantified
557 using real-time qPCR and next-generation mRNA sequencing. Primer information for qPCR
558 is described in Supplementary Table 13. A total of 60 samples was sequenced, consisting of 5
559 biological replicates at four different time points (6, 13, 120 dpf, and acute-stressed at 120
560 dpf) for each genotype (wild type, bPAC+ and bPAC-).
561
562 **Immunohistochemistry**

Immunohistochemistry was performed as described with minor modifications(79). Custom-made primary rabbit anti-oxt antibodies (1:200 dilution)(79) and Alexa 488 anti-rabbit antibodies (A-11008, Invitrogen, USA) as secondary antibodies (1:1000 dilution) were used for the visualization of oxytocinergic neurons.

Imaging and analysis

Confocal stacks through the larval head were recorded using a Zeiss LSM 880 with Airyscan FAST with PApo 10X/0.45 – dry objective with Airyscan mode. Stacks were analyzed using ImageJ (FIJI, 1.53c)(80). Corrected Total Cell Fluorescence (CTCF) was calculated as integrated density − (Area of cells × Mean fluorescence of background reading).

Statistics

Statistical analyses were performed using R and Prism 9 (Graphpad Software Inc, San Diego, CA, USA). A detailed description of the statistical analyses is provided in online methods(78)

Data availability

All sequenced reads for RNA-seq were deposited in European Nucleotide Archive (ENA, PRJEB53713).

Code availability

The detailed data processing and R scripts for bioinformatic analysis are available at the GitHub repository (https://github.com/minkechoi/tx_star-bPAC_brain).

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Author contributions

SR conceived the project. MC and SR designed the project. MC acquired and analyzed transcriptomic data. AC acquired and analyzed behavior data. SK, HE, AT, and SK contributed to reagent generation or data acquisition. MC, SR, and AC drafted the article. MC, HE, and SR critically revised the manuscript. All authors approved the submission.

Competing interests

SR holds a patent, European patent number 2928288 and US patent number 10,080,355: "A novel inducible model of stress.". The remaining authors declare no known competing interests.

608 **Figure legends**

609 **Figure 1. Ambient light-exposed bPAC+ fish show elevation of cortisol during**
610 **development.** (a) An image of *Tg(star:bPAC-2A-tdTomato)*$^{+/-}$ larva at 5 dpf. The transgene is
611 expressed solely in the interrenal gland (arrow). The schematic represents the intracellular
612 events in the steroidogenic interrenal cells of transgenic fish (dotted box). Endogenous GC
613 production is stimulated by ACTH signaling. Similarly, bPAC mimics ACTH signaling by
614 increasing cAMP when exposed to blue light. (b) Fish were raised under ambient aquarium
615 lighting conditions with white fluorescent light (12-hour light and 12-hour dark). Elevation of
616 basal GC level was observed at larval and juvenile stages. (c) White ambient light (blue+)
617 elevates the basal GC level of 6 dpf bPAC+ larvae. ***=P<0.001, two-tailed unpaired t-test.
618 However, there was no significant difference in GC level when fish were raised under filter
619 restricting blue light spectrum within the ambient light (blue-). (d) One of the well-known GR
620 signaling markers, *fkbp5*, was significantly upregulated in bPAC+ larvae (P = 0.016) compared
621 to wild-type control, two-tailed unpaired t-test. * = P<0.05. Basal GC level at larval stages (e)
622 and from juvenile to adult (f). Abbreviations: GC, glucocorticoid; ACTH, Adrenocorticotropic
623 Hormone; AC, adenylyl cyclase; cAMP, Cyclic adenosine monophosphate
624
625 **Figure 2. Reduced social interaction in adult bPAC+ fish.** No significant differences were
626 observed in average speed (a) nor turn frequency (b) among wild type, bPAC+ and bPAC-. (c)
627 Schematic figures for the social approach and maintenance test. During the pre-social phase,
628 no significant difference in color preference (d) nor depth (e) was observed. (f) Representative
629 traces of wild-type fish during the social phase. The green shade indicates the interaction zone.
630 (g) During the social phase, no significant difference in approach rate to conspecific fish
631 between groups. Log-rank test (Mantel-Cox, P = 0.77). (h) Representative traces of wild-type
632 and bPAC+ fish during the social phase. bPAC+ and bPAC- showed a significantly reduced
633 duration (i) and frequency of social interactions (j) compared to the wild type. Dunnett's
634 multiple comparisons followed one-way ANOVA, a) F(2, 27)=0.2248, P=0.80; b) F(2,
635 27)=2.332, P=0.12; d) F(2, 98)=0.6224, P=0.54; e) F(2, 98)=1.801, P=0.1706; i) F(2,
636 98)=6.273, P=0.0027; j) F(2, 98)=10.93, P<0.0001.
637
638 **Figure 3. Developmental GC exposure leads to differential gene expressions across the**
639 **life course** (a) Venn Diagram for up- and down-regulated DEGs between wild type and bPAC+;
640 criteria for DEG identification: |FC|>2 and FDR <0.01. A lower FC threshold, |FC|>1.5, was
641 applied for samples at 120 dpf. (b) Average read counts of genes by genotype and time points
642 are represented by z-score in the heat map. Bars on the right side indicate the paternity of fish.
643 (c) Expression profiles of GC-associated genes. *=P<0.05, **=P<0.01, ***=P<0.001,
644 ****=P<0.0001. Tukey's multiple comparisons test followed two-way repeated measures
645 ANOVA. (d) Top enriched biological processes for up- and down-regulated DEGs in bPAC+.
646 d6: 6 dpf, d13: 13 dpf, d120: 120 dpf, wt: wild type, FC: fold change, GC: glucocorticoid, *adj.p*:
647 adjusted P-value, ratio: identified DEGs/the number of genes included in the term. Complete
648 DEG-enriched GO terms are described in Supplementary Table 1.
649
650 **Figure 4. Developmental GC exposure primes differential gene expression following acute**
651 **stress in bPAC+ adults.** (a) Schematic of the acute stress paradigm. Looming dots (LD) mimic
652 approaching predators from above (Cook et al. 2023). (b) Schematic for acute-stressed and
653 control sample collection (arrows). Acute stress is induced by looming dot (LD)-exposure in
654 adults (120 dpf). post-LD samples were collected after 15 min of LD exposure. Yellow striped
655 box: bPAC+ activation; yellow stars: LD. (c) bPAC+ showed significantly higher GC levels in
656 post-LD conditions, compared with basal. Tukey's multiple comparisons followed one-way
657 ANOVA; F(5, 54)=19.31, P<0.0001. (d) Expression profiles of GC-induced genes following

658 LD exposure. *= $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ from multiple two-tailed unpaired
659 t-tests. Error bars indicate mean+SEM. (e) Schematic for three comparisons to identify
660 transcriptional changes in response to LD exposure in adults. DEGs were determined by FC
661 and FDR. |FC|>1.5 and FDR<0.01 for DEG1 and 2; |FC|>2 and FDR<0.01 for DEG3. (f) z-
662 score for mean CPM of Adult bPAC+ DEGs is visualized in a heatmap. (g) Venn diagram for
663 Adult bPAC+ DEGs. The list of DEGs is described in Supplementary Table 2. The Adult
664 bPAC+ DEGs = DEG1 + DEG2 + DEG3 - (DEG1 ∩ DEG2). GC-primed genes = DEG1 +
665 DEG2 - (DEG1 ∩ DEG2). The red asterisk represents the overlap (2 genes) between DEG1
666 and DEG3. (h) The top 3 GO terms enriched in downregulated GC-primed genes. The
667 enrichment score = $-\log_{10}$(adjusted *P*-value). The complete results of the functional enrichment
668 test are described in Supplementary Table 3.
669
670 **Figure 5. Adult bPAC+ DEGs are enriched in gene sets associated with human**
671 **neuropsychiatric disorders.** (a) Result of human disorders enrichment test based on the
672 DisGeNET database. The ratio represents overlapping/input genes. Top ranked disorders are
673 schizophrenia (FDR= 1.61e-04, ratio (Adult bPAC+ DEGs/disease genes)=170/883), bipolar
674 disorder (FDR= 7.48e-04, ratio=100/477), depressive disorder (FDR= 2.89e-03, ratio=63/289),
675 mental depression (FDR= 2.87e-03, ratio=59/254) and autistic disorder (FDR= 1.61e-04,
676 ratio=65/261). (b) Venn diagram for Adult bPAC+ DEGs that are associated with the top five
677 human psychiatric disorders in panel a. Depressive disorder and Mental Depression-associated
678 genes (as shown in a), are combined and referred to as depressive disorders in b and d. (c)
679 STRING protein-protein interaction (PPI) networks of schizophrenia-associated Adult bPAC+
680 DEGs genes in pre- and post-LD conditions indicate LD-sensitive gene expression. The size
681 and color of the node represent comparative fold change, and gene expression; up (orange) or
682 downregulation (blue), respectively. DEGs enriched in KEGG pathways Neuroactive ligand-
683 receptor interaction (FDR = 2.01e-08) are labeled in yellow whilst those enriched in MAPK
684 signaling pathway (FDR=9.74e-08) are labeled in green. (d) Top GO terms enriched in the top-
685 ranked disease-associated Adult bPAC+ DEGs. Adult bPAC+ DEGs that are associated with
686 schizophrenia, depression, autism, and bipolar disorder are mainly enriched in the
687 downregulated functions in synaptic signaling, and multicellular organismal process. The
688 enrichment score = -log10(adjusted P-value). (e) The heatmap depicts the $\log_2$FC of GC-altered
689 genes associated with the top five human psychiatric disorders in panel a (Depressive disorder
690 and mental depression are merged). The numbers in the brackets represent the number of Adult
691 bPAC+ DEGs. 6, 13, and 120 denote dpf, and LD denotes post-LD at 120 dpf. (f) Subsets of
692 Adult bPAC+ DEGs associated with the top five diseases in a show consistent differential
693 expression across the life course (significant at all time points, labeled trans-temporal) or only
694 in adulthood (significant only in adulthood, labeled adult-onset). Genes that exhibited
695 expression patterns outside of these conditions were excluded.
696
697 **Figure 6. Social behavior-associated genes tend to be downregulated in bPAC+ fish across**
698 **the life course.** (a) The expression of social behavior-associated genes, log2FC from two
699 comparisons, bPAC+ vs. wild type, and bPAC- vs. wild type are represented by a heatmap.
700 Asterix in each cell denotes that FDR < 0.05. Red labels indicate GC-primed genes. (b)
701 Decreased *oxt* expression in bPAC+ was confirmed both in larval and adult brains by real-time
702 qPCR. * = $P<0.05$, multiple unpaired t-tests. Error bar indicates mean±SEM. (c) Expression of
703 *oxt* was decreased especially in the medial posterior cluster of oxt+ cells (yellow arrows) in the
704 neurosecretory preoptic area (NPO) of 5 dpf bPAC+ larvae. a: anterior, p: posterior. scale bar
705 represents 25μm. (d) the signal intensity of *oxt* in NPO was significantly reduced in bPAC+
706 NPO. (e) Log2FC of known *oxt* regulatory elements. *= FDR < 0.05. (f) Top enriched
707 transcription factors for differentially expressed social behavior-associated genes. Only

708 differentially expressed TFs at any time point in this study were represented. Light green
709 represents TFs that are significantly differentially expressed in bPAC+.
710

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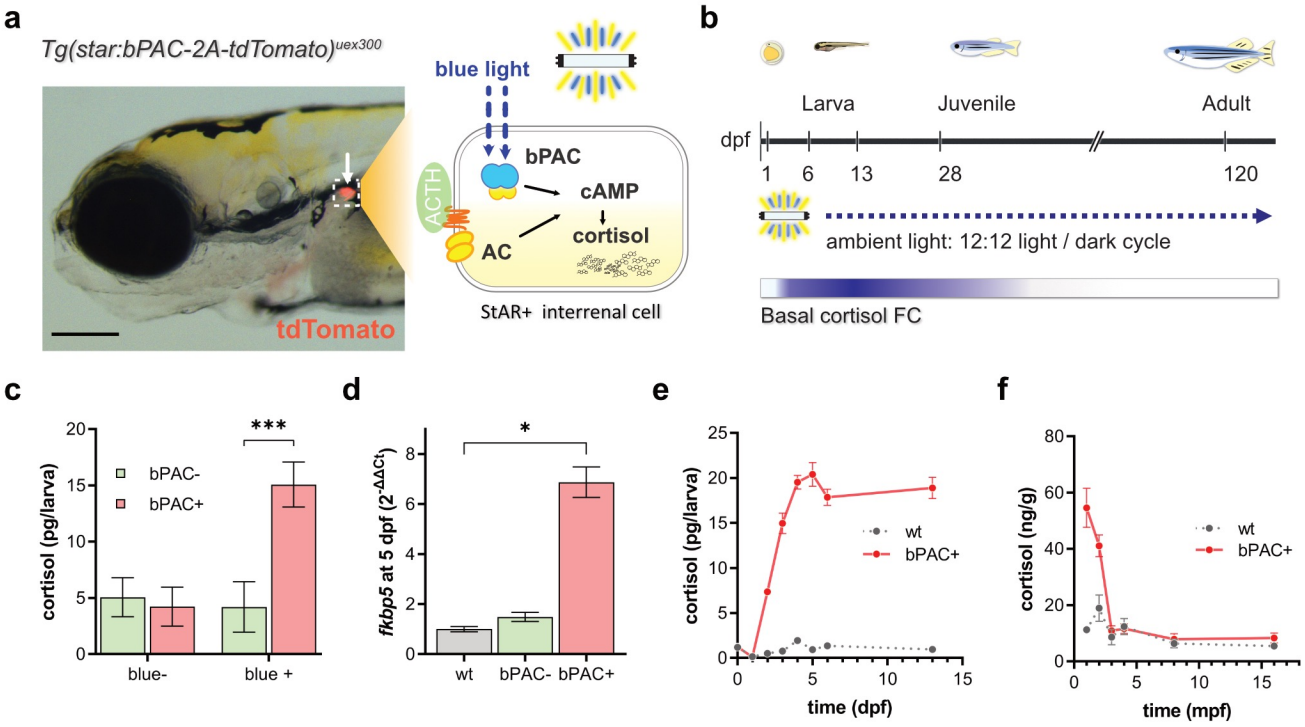

a
Tg(star:bPAC-2A-tdTomato)uex300
tdTomato
blue light
bPAC
cAMP
cortisol
AC
ACTH
StAR+ interrenal cell
b
Larva
Juvenile
Adult
dpf
1
6
13
28
120
ambient light: 12:12 light / dark cycle
Basal cortisol FC
c
cortisol (pg/larva)
bPAC-
bPAC+
***
blue-
blue +
d
fkbp5 at 5 dpf (2-ΔΔCt)
*
wt
bPAC-
bPAC+
e
cortisol (pg/larva)
wt
bPAC+
time (dpf)
f
cortisol (ng/g)
wt
bPAC+
time (mpf)

Figure 1

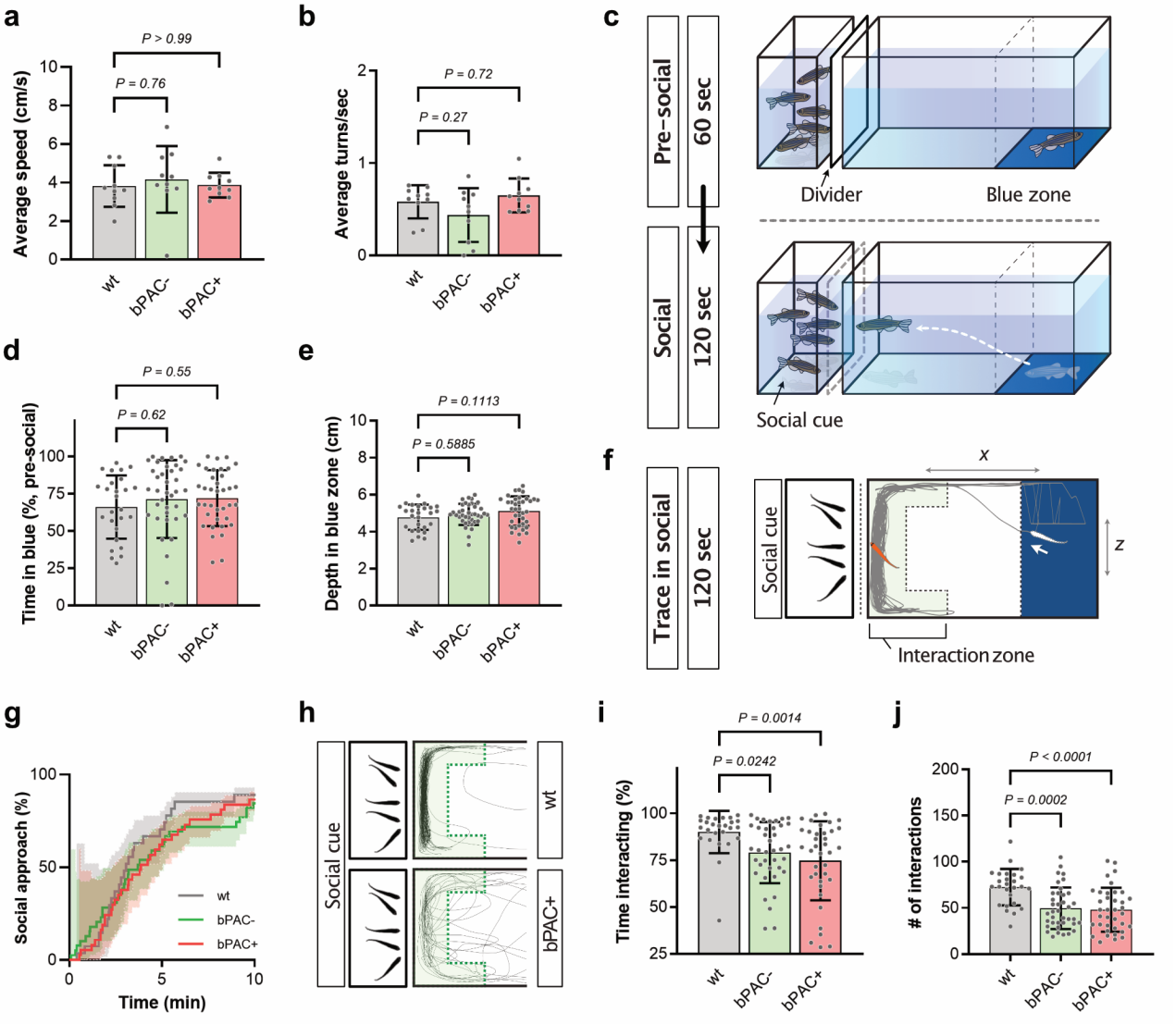

a
Average speed (cm/s)
P > 0.99
P = 0.76
wt
bPAC-
bPAC+
b
Average turns/sec
P = 0.72
P = 0.27
c
Pre-social
60 sec
Divider
Blue zone
Social
120 sec
Social cue
d
Time in blue (%, pre-social)
P = 0.55
P = 0.62
e
Depth in blue zone (cm)
P = 0.1113
P = 0.5885
f
Trace in social
120 sec
Social cue
x
z
Interaction zone
g
Social approach (%)
Time (min)
wt
bPAC-
bPAC+
h
Social cue
wt
bPAC+
i
Time interacting (%)
P = 0.0014
P = 0.0242
j
# of interactions
P < 0.0001
P = 0.0002

Figure 2

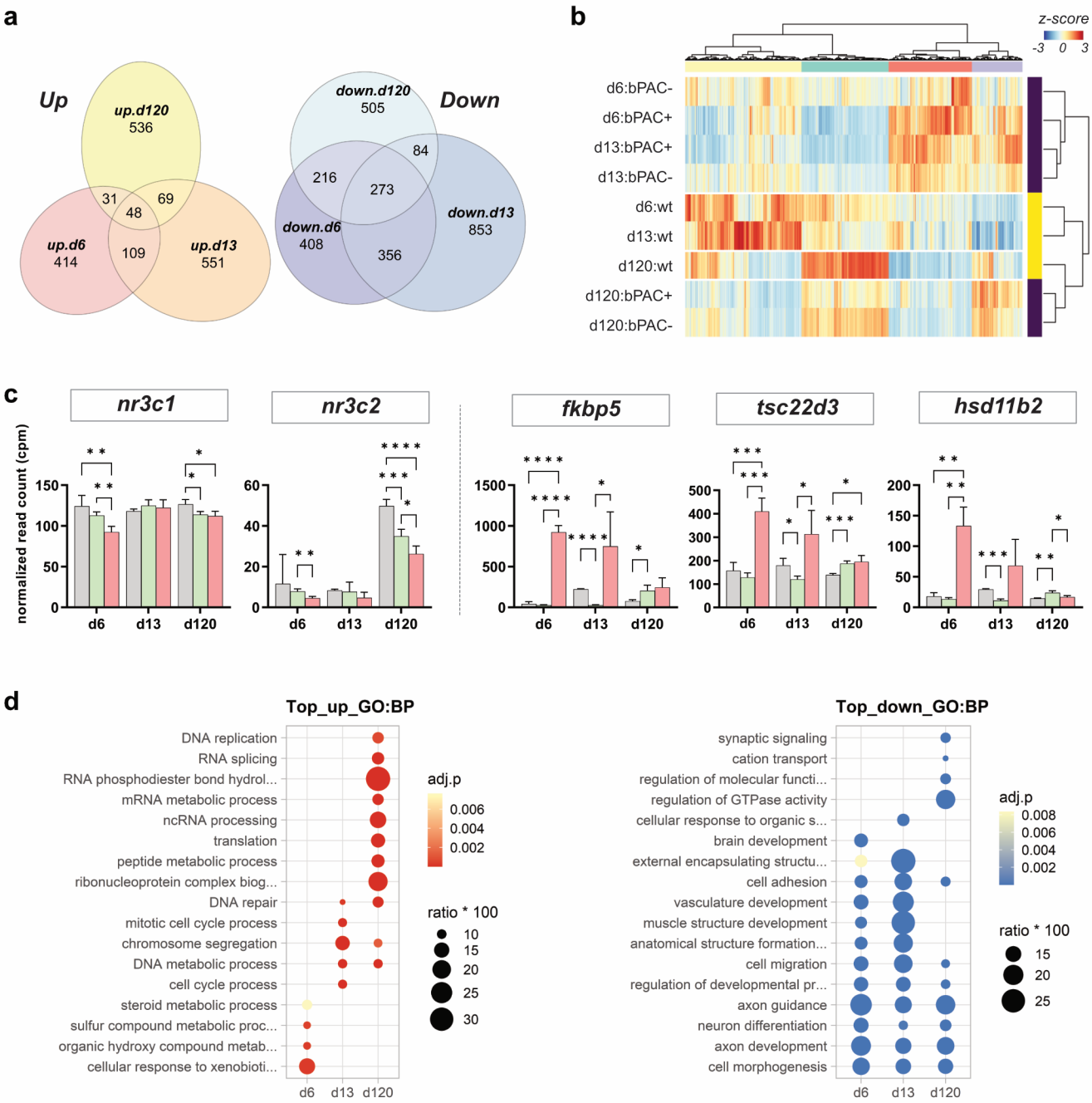

a
Up
up.d120
536
up.d6
414
up.d13
551
31
69
48
109
Down
down.d120
505
down.d6
408
down.d13
853
84
216
273
356
b
z-score
-3 0 3
d6:bPAC-
d6:bPAC+
d13:bPAC+
d13:bPAC-
d6:wt
d13:wt
d120:wt
d120:bPAC+
d120:bPAC-
c
nr3c1
nr3c2
fkbp5
tsc22d3
hsd11b2
normalized read count (cpm)
d6
d13
d120
d
Top_up_GO:BP
DNA replication
RNA splicing
RNA phosphodiester bond hydrol...
mRNA metabolic process
ncRNA processing
translation
peptide metabolic process
ribonucleoprotein complex biog...
DNA repair
mitotic cell cycle process
chromosome segregation
DNA metabolic process
cell cycle process
steroid metabolic process
sulfur compound metabolic proc...
organic hydroxy compound metab...
cellular response to xenobioti...
adj.p
0.006
0.004
0.002
ratio * 100
10
15
20
25
30
Top_down_GO:BP
synaptic signaling
cation transport
regulation of molecular functi...
regulation of GTPase activity
cellular response to organic s...
brain development
external encapsulating structu...
cell adhesion
vasculature development
muscle structure development
anatomical structure formation...
cell migration
regulation of developmental pr...
axon guidance
neuron differentiation
axon development
cell morphogenesis
adj.p
0.008
0.006
0.004
0.002
ratio * 100
15
20
25

Figure 3

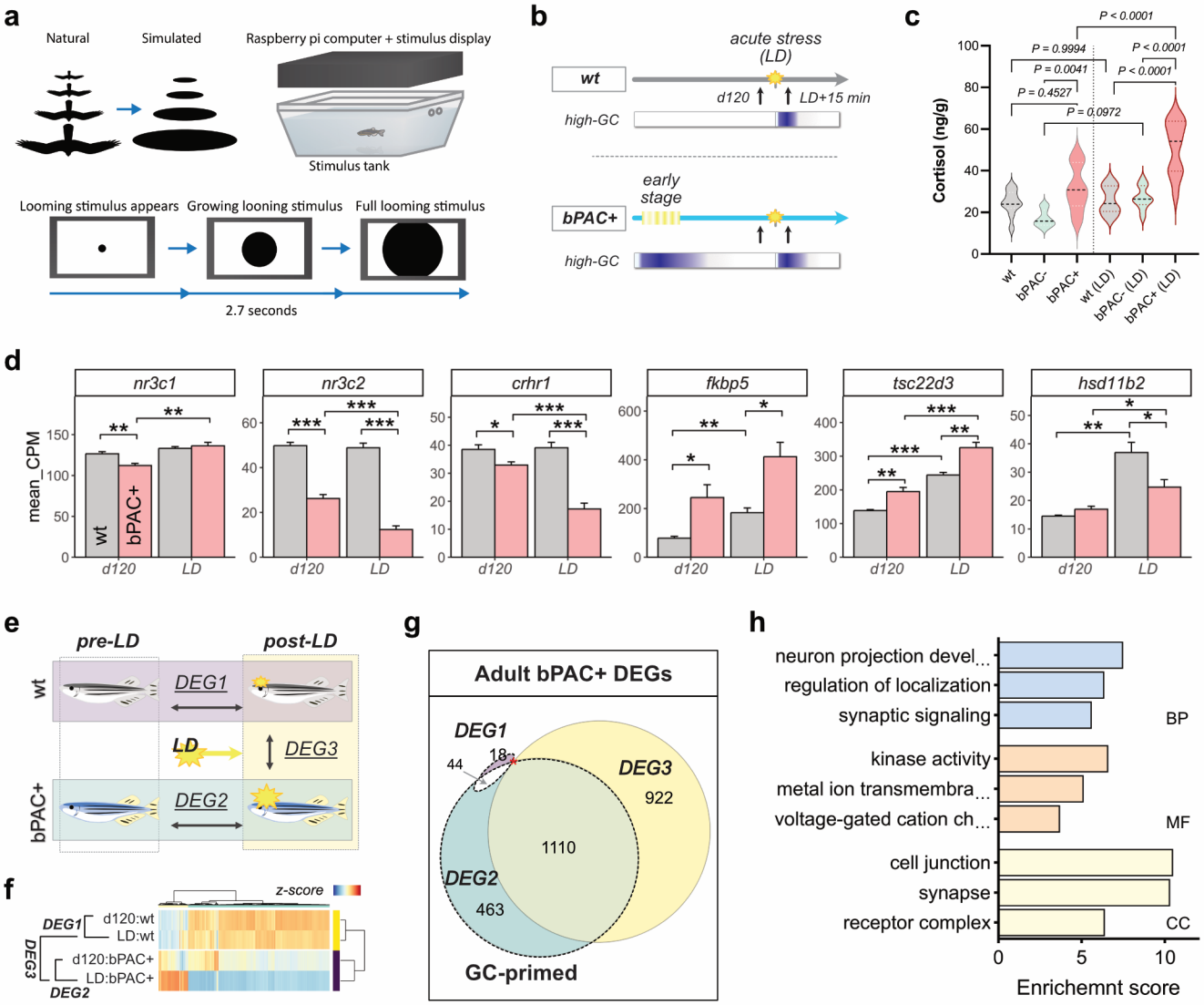
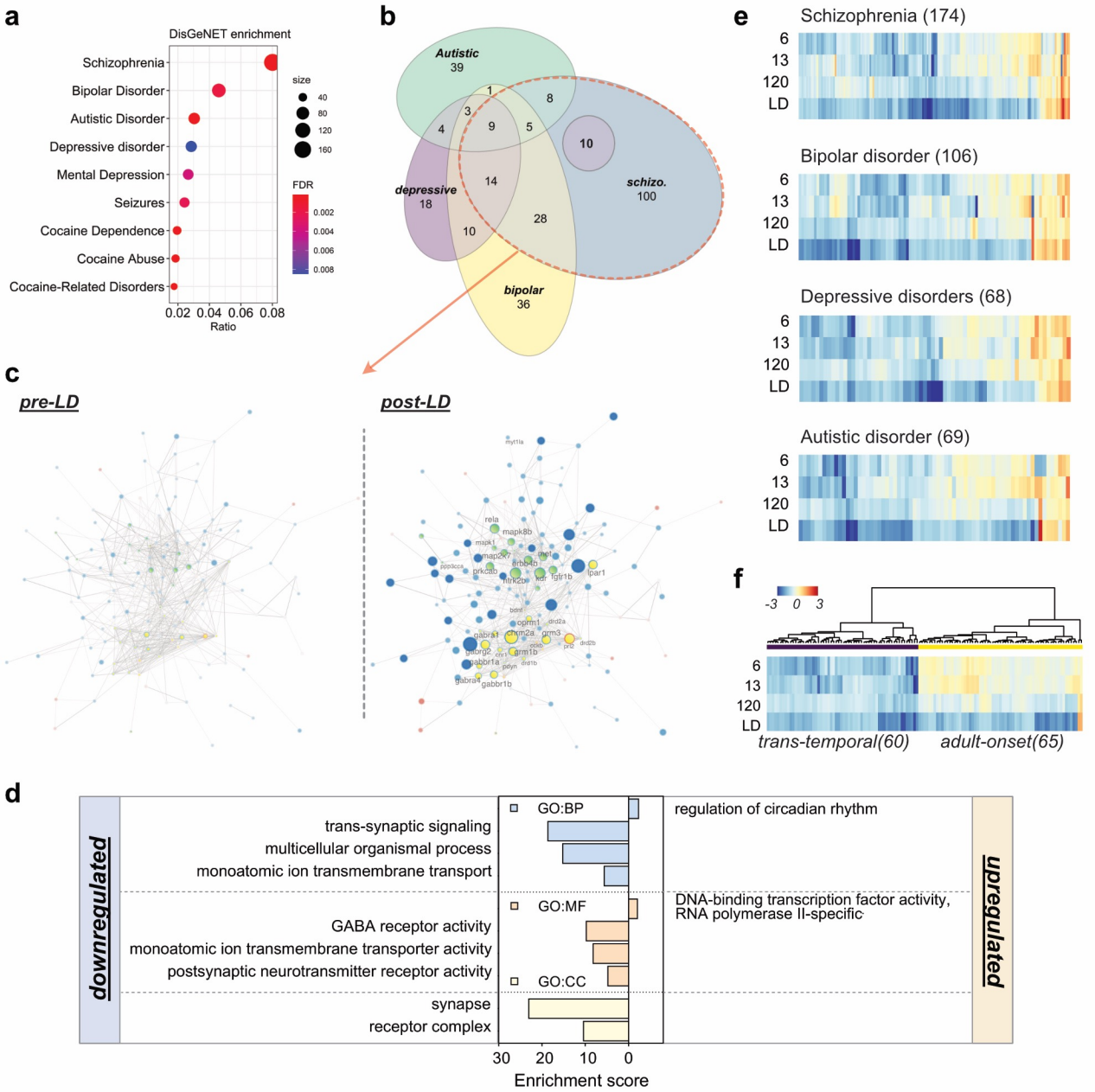

a
Natural
Simulated
Raspberry pi computer + stimulus display
Stimulus tank
Looming stimulus appears
Growing looning stimulus
Full looming stimulus
2.7 seconds
b
acute stress (LD)
wt
d120
LD+15 min
high-GC
early stage
bPAC+
high-GC
c
Cortisol (ng/g)
P = 0.9994
P < 0.0001
P = 0.0041
P < 0.0001
P = 0.4527
P = 0.0972
P < 0.0001
wt
bPAC-
bPAC+
wt (LD)
bPAC- (LD)
bPAC+ (LD)
d
mean_CPM
nr3c1
nr3c2
crhr1
fkbp5
tsc22d3
hsd11b2
wt
bPAC+
d120
LD
e
pre-LD
post-LD
wt
DEG1
LD
DEG3
bPAC+
DEG2
f
z-score
DEG1
d120:wt
LD:wt
DEG3
d120:bPAC+
LD:bPAC+
DEG2
g
Adult bPAC+ DEGs
DEG1
44
18
DEG3
922
1110
DEG2
463
GC-primed
h
neuron projection devel...
regulation of localization
synaptic signaling
BP
kinase activity
metal ion transmembra...
voltage-gated cation ch...
MF
cell junction
synapse
receptor complex
CC
Enrichemnt score

Figure 4


a
DisGeNET enrichment
Schizophrenia
Bipolar Disorder
Autistic Disorder
Depressive disorder
Mental Depression
Seizures
Cocaine Dependence
Cocaine Abuse
Cocaine-Related Disorders
0.02 0.04 0.06 0.08
Ratio
size
40
80
120
160
FDR
0.002
0.004
0.006
0.008
b
Autistic
39
depressive
18
schizo.
100
bipolar
36
1
8
3
4
9
5
10
14
28
10
c
pre-LD
post-LD
e
Schizophrenia (174)
Bipolar disorder (106)
Depressive disorders (68)
Autistic disorder (69)
6
13
120
LD
f
-3 0 3
6
13
120
LD
trans-temporal(60)
adult-onset(65)

d
downregulated
upregulated
GO:BP
trans-synaptic signaling
multicellular organismal process
monoatomic ion transmembrane transport
regulation of circadian rhythm
GO:MF
GABA receptor activity
monoatomic ion transmembrane transporter activity
postsynaptic neurotransmitter receptor activity
DNA-binding transcription factor activity,
RNA polymerase II-specific
GO:CC
synapse
receptor complex
30 20 10 0
Enrichment score

Figure 5

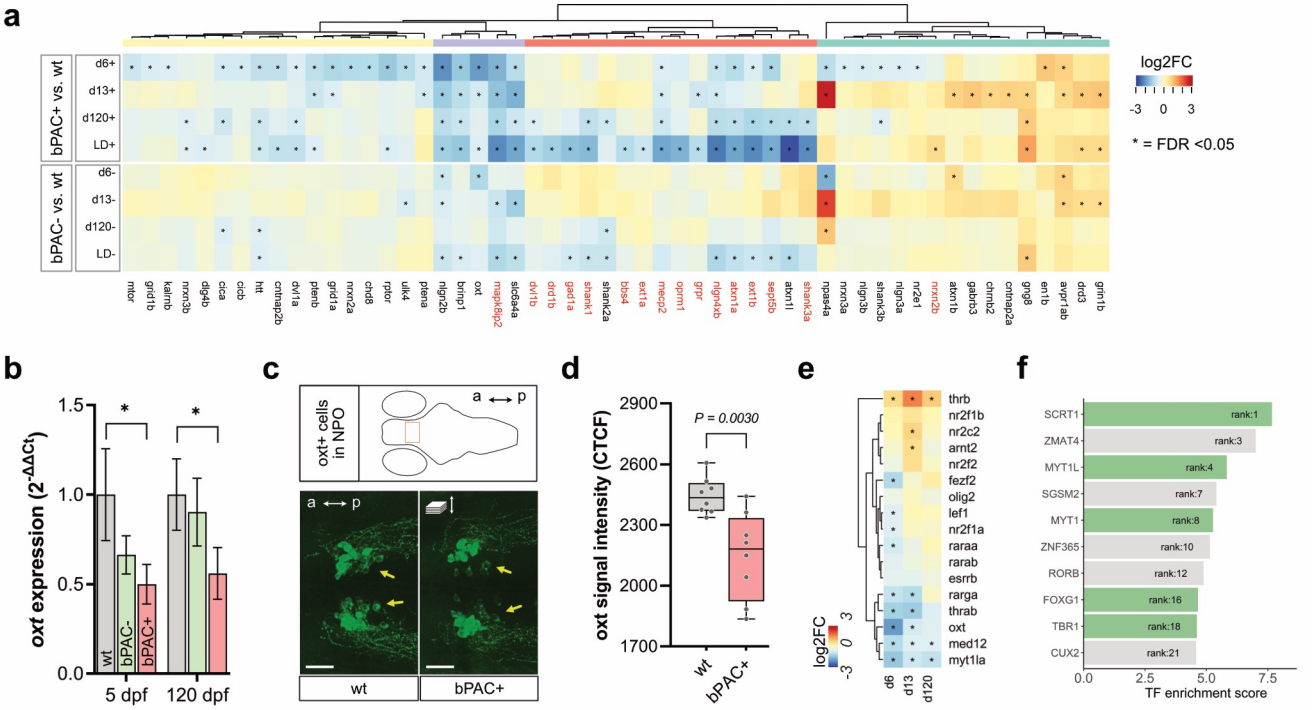

a
bPAC+ vs. wt
bPAC- vs. wt
d6+
d13+
d120+
LD+
d6-
d13-
d120-
LD-
log2FC
-3 0 3
* = FDR <0.05
mtor
grid1b
kalrnb
nrxn3b
dlg4b
cica
cicb
htt
cnthap2b
dvl1a
ptenb
grid1a
nrxn2a
chd8
rptor
ulk4
ptena
nlgn2b
brnp1
oxt
mapk8ip2
slc6a4a
dvl1b
drd1b
gad1a
shank1
shank2a
bbs4
ext1a
mecp2
oprm1
grpr
nlgn4xb
atxn1a
ext1b
sept5b
atxn1l
shank3a
npas4a
nrxn3a
nlgn3b
shank3b
nlgn3a
nr2e1
nrxn2b
atxn1b
gabrb3
chrnb2
cnthap2a
gng3
en1b
avpr1ab
drd3
grin1b
b
oxt expression (2^-ΔΔCt)
1.5
1.0
0.5
0.0
*
*
wt
bPAC-
bPAC+
5 dpf
120 dpf
c
oxt+ cells in NPO
a ⟷ p
a ⟷ p
wt
bPAC+
d
oxt signal intensity (CTCF)
2900
2600
2300
2000
1700
P = 0.0030
wt
bPAC+
e
log2FC
3
0
-3
d6
d13
d120
thrb
nr2f1b
nr2c2
arnt2
nr2f2
fezf2
olig2
lef1
nr2f1a
raraa
rarab
esrrb
rarga
thrab
oxt
med12
myt1la
f
SCRT1
ZMAT4
MYT1L
SGSM2
MYT1
ZNF365
RORB
FOXG1
TBR1
CUX2
rank:1
rank:3
rank:4
rank:7
rank:8
rank:10
rank:12
rank:16
rank:18
rank:21
0.0
2.5
5.0
7.5
TF enrichment score

Figure 6