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Atomoxetine Reduces Anticipatory Responding in a 5-Choice Serial Reaction
Time Task For Adult Zebrafish

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Abstract

Deficits in impulse control are related to a number of psychiatric diagnoses, including ADHD, addiction and pathological gambling. Despite increases in our knowledge about the underlying neurochemical and neuroanatomical correlates, understanding of the molecular and cellular mechanisms is less well established. Understanding these mechanisms is essential in order to move towards individualized treatment programs and increase efficacy of interventions. Zebrafish are a very useful vertebrate model for exploring molecular processes underlying disease owing to their small size and genetic tractability. Their utility in terms of behavioral neuroscience, however, hinges on the validation and publication of reliable assays with adequate translational relevance. Here we report an initial pharmacological validation of a fully automated zebrafish version of the commonly used 5-choice serial reaction time task (5-CSRTT) using a variable interval (VI) pre-stimulus interval (PSI). We found that atomoxetine reduced anticipatory responses (0.6 mg/Kg), while a high dose (4 mg/Kg) methylphenidate increased anticipatory responses and the number of trials completed in a session. On the basis of these results, we argue that similar neurochemical processes in fish as in mammals may control impulsivity, as operationally defined by anticipatory responses on a continuous performance task such as this, making zebrafish potentially a good model for exploring the molecular basis of impulse control disorders, and for first-round drug screening.

Keywords: 5-choice serial reaction time task, zebrafish, impulsivity, addiction, ADHD, atomoxetine, methylphenidate

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73**Introduction**

Impulsivity, as operationally defined in terms of anticipatory responding on a continuous performance task, has been linked to a number of psychiatric diagnoses, including attention deficit hyperactivity disorder (ADHD) (Urcelay and Dalley 2012; Winstanley et al. 2006), substance abuse (Dalley et al. 2011; Everitt et al. 2008; Hosking and Winstanley 2011) and pathological gambling (Alessi and Petry 2003). Despite a recent increase in our understanding of the neurochemical and neuroanatomical correlates of impulsivity (Caprioli et al. 2013; Dalley and Roiser 2012), the underlying cellular processes are somewhat less clear.

Zebrafish provide an excellent model for studying the molecular basis of human disease owing to their prolific breeding, low maintenance costs and genetic tractability (Guo 2004; Parker and Brennan 2012; Parker et al. 2013a). We previously demonstrated that adult zebrafish perform well in terms of their general response characteristics (accuracy, anticipatory responding, omissions) on a 3-choice (Parker et al. 2012a) and later a 5-choice version (Parker et al. 2013b) of the commonly used 5-CSRTT (Carli et al. 1983; Robbins 2002) for rodents.

Impulsivity, as operationalized by the rate of anticipatory responding on the task, is a strong predictor for compulsive drug seeking (Belin et al. 2008) and relapse following withdrawal from drugs (Economidou et al. 2009). Understanding the cellular and molecular basis of impulsivity may help us to develop individualized treatment for recovering addicts, but also potentially to design early interventions for at-risk individuals.

In the present paper, we carried out an initial pharmacological validation of the 5-CSRTT in adult zebrafish using drugs that have previously been shown to affect rodents' performance on the task with well-defined and frequently replicated results. Methylphenidate is a dopamine and noradrenaline reuptake inhibitor, and has long been used to treat the symptoms of ADHD

74 (Barkley 1997), but its effects on anticipatory responding in the 5-CSRTT are less clear with
75 some studies showing increases in anticipatory response, and some decreases, at various doses
76 (Bizarro et al. 2004; Navarra et al. 2008). Atomoxetine (Tomoxetine hydrochloride, LY 139603)
77 is a selective noradrenaline reuptake inhibitor, which has also been successfully used in the
78 treatment symptoms of ADHD (Michelson et al. 2001). Atomoxetine has shown high efficacy in
79 reducing anticipatory responding on the 5-CSRTT in rodents (Economidou et al. 2011;
80 Economidou et al. 2012; Fernando et al. 2012; Robinson et al. 2008). We incubated adult
81 zebrafish in different doses of each of the drugs prior to probing anticipatory response rates on
82 the 5-CSRTT using variable interval (VI) pre-stimulus intervals (PSI).

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Method

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Subjects

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Nineteen adult, mixed-sex, wild-type (TU strain) zebrafish were bred in our aquarium facility at Queen Mary University of London (QMUL), and reared up to four months of age according to established protocols (Westerfield 1993). At four months, the fish were moved into our behavioral testing facility and pair-housed (26-28°C, 160 lx ambient lighting; 14/10 hr light/dark cycle) for 1-week prior to commencing the experiment. They remained pair housed throughout the experimental period. Throughout the experiment, all fish were fed live brine shrimp and flake food at weekends, and brine shrimp liquidized with bloodworm during testing (see below) supplemented with commercial dried flake food in the evening after testing. All procedures were carried out in accordance with the Animals (Scientific Procedures) Act of 1986, and local ethical guidelines.

97 **Apparatus**

98 [FIGURE 1 ABOUT HERE]

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100 The fish were trained in a custom-built testing arena (Figure 1) manufactured in-house at
101 QMUL (Parker et al. 2013b). Briefly, the entire length of the testing unit was 36cm, split into
102 two halves by the gate (21cm from food area to gate, 15cm from gate to stimulus areas). The gate
103 is used in order to signal the start and end of trials, and to ensure that all of the fish start each
104 trial from the same vantage point. In the rodent version of the task, the box is smaller in
105 comparison to the size of the animal. We have attempted to use a smaller box in previous
106 implementations of this task, but the fish do not perform well if confined to small spaces. The
107 external tank (W x L x H: 42cm x 49cm x 15cm) was purchased commercially (Ikea, UK). The
108 base was constructed from 10mm clear cast acrylic and drilled to fix two uprights to support the
109 gate mechanism. The testing unit was constructed from opaque acrylic, and a 96-channel i-o card
110 drove the actuators (National Instruments, Austin, TX). The apparatus were controlled via a
111 program written in LabView (National Instruments, Austin, TX) that also collected the data
112 during training sessions. The gate was operated via a pneumatic cylinder (RS Components, UK).
113 The movements of the fish in the tank, and hence the actuation of the hardware, was performed
114 by a custom-written (Python) camera-based fish detection system. The cameras were located
115 above the tanks (Windows LifeCam HD). Food delivery was controlled by a linear stepper motor
116 (RS Components, UK), calibrated to deliver $\sim 10\mu\text{l}$ liquidized bloodworm/brine shrimp mixture
117 via a syringe and a length of 1mm catheter tubing. The stimuli at the stimulus end of the tank
118 comprised five super-bright yellow LEDs (RS components, UK) and the stimulus in the
119 magazine area comprised a single super-bright green LED.

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120 Atomoxetine (Tomoxetine hydrochloride, Tocris Bioscience, Bristol, UK; $0.5\mu\text{M}$
121 [0.15mg/Kg], $1\mu\text{M}$ [0.3mg/Kg], and $2\mu\text{M}$ [0.6mg/Kg]) and methylphenidate (Threo-
122 methylphenidate hydrochloride, Tocris Bioscience, Bristol, UK; $5\mu\text{M}$ [1.3mg/Kg], $10\mu\text{M}$
123 [2.6mg/Kg], and $15\mu\text{M}$ [4mg/Kg]) were dissolved in aquarium-treated water and administered to
124 each fish at three different doses via incubation in the drug solution for 30-minutes prior to
125 testing. The incubation tank was a 1-liter transparent acrylic tank, identical to the fishes' housing
126 tanks, located adjacent to the testing tanks.

127

128 **Procedure**

129 Prior to training, all fish were acclimated to the behavioral testing room for one week
130 (Week 0). All testing sessions lasted for 30-minutes, and were carried out Monday-Friday. The
131 time of day that the fish were tested was staggered to avoid potential diurnal performance
132 confounds, but the tank in which each fish was tested remained the same for every session. In the
133 first week of pre-training (Week 1), the fish were habituated to the testing tanks. During this
134 time, all of the lights remained illuminated and the gate was raised. Food was delivered
135 intermittently according to a 1-minute fixed time (FT) schedule following entry to the food
136 magazine. In the second week of pre-training (Week 2), the fish were 'magazine trained'.
137 During this phase, the gate was closed and the fish was isolated in the food-delivery end of the
138 tank. The magazine light was illuminated for up to 30-seconds (1-minute inter-trial interval; ITI),
139 or until the fish entered the food magazine. Correct entries (i.e., entry during the stimulus
140 exposure) were reinforced in a discrete trial manner (see above). Entries during the ITI were
141 neither reinforced nor punished. In the third and final week of pre-training (Week 3), the fish
142 were trained to approach the stimulus lights at the far end of the tank. At the start of a session,

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143 the fish was isolated in the food delivery area of the tank, with the magazine light illuminated.
144 Entry to the magazine started the session. After an ITI of 20-seconds, the gate was raised to
145 reveal the stimulus apertures. All LEDs were illuminated contiguously for 1-minute. During this
146 time, entry to any of the stimulus apertures was conditionally reinforced by illumination of the
147 magazine light. As the fish swam past the gate it was lowered, and entry to the food magazine
148 was reinforced. The following trial began after a 20-second ITI. Late entries were not reinforced
149 or punished, but the fish was isolated in the food delivery area following re-entry for a 20-second
150 ITI. The fish were then trained on the 5-CSRTT. The general procedure was as in Week 3, but
151 only one stimulus light was illuminated at any one time, and we introduced a pre-stimulus
152 interval (PSI), which represented the delay between the gate being raised and the stimulus being
153 illuminated.

154 Training was split into three distinct phases. The criterion for moving from each phase to
155 the next was that the fish performed ≥ 20 trials in each session for a minimum of three
156 consecutive days. In the first phase (weeks 4-5), the stimulus duration was 30-seconds, and the
157 pre-stimulus interval (PSI) was 1-second (FI schedule). In the second phase (weeks 6-9), the
158 stimulus duration remained at 30-seconds, but the pre-stimulus interval changed to a 5-second
159 variable interval (VI) schedule. The third phase (weeks 10-15) incorporated the drug trials.
160 Atomoxetine was administered at $0.5\mu\text{M}$, $1\mu\text{M}$, and $2\mu\text{M}$, and methylphenidate at $5\mu\text{M}$, $10\mu\text{M}$,
161 and $15\mu\text{M}$. These dose ranges were based on previous work with rodents (Bizarro et al. 2004;
162 Economidou et al. 2011; Fernando et al. 2012; Milstein et al. 2010; Navarra et al. 2008;
163 Robinson et al. 2008) and with zebrafish (Lange et al. 2012). During each drug treatment week,
164 the treatment schedule was as follows: Monday – baseline; Tuesday – drug; Wednesday-
165 Thursday – baseline; Friday – drug. This allowed for a minimum of two days of washout

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166 between drug treatments. Each fish received all doses of both drugs twice during the course of
167 the experiment, with each fish receiving the same drug twice in the same week. The order in
168 which the drugs and doses were given was counterbalanced between fish to avoid any possibility
169 of order effects. Performance parameters were calculated as thus:

$$170 \quad \textit{accuracy} = \textit{correct}/(\textit{correct} + \textit{incorrect})$$

$$171 \quad \textit{anticipatory} = \textit{early}/(\textit{correct} + \textit{incorrect} + \textit{early})$$

$$172 \quad \textit{omissions} = \textit{omissions}/(\textit{correct} + \textit{incorrect} + \textit{early} + \textit{omissions})$$

173

174 Finally, data were analyzed using general or generalized linear mixed effects models
175 (LME), fit by restricted maximum likelihood (REML) with drug as a fixed effect with seven
176 levels (Baseline, Methylphenidate: 5 μ M, 10 μ M, 15 μ M, Atomoxetine: 0.5 μ M, 1 μ M, 2 μ M), and
177 fish ID (random intercept) and day as scalar random effects, followed by pairwise comparisons
178 (Least Significant Difference; LSD). We used two drug days for each fish specifically as the
179 fishes' performance on the task is far more variable than that of rodents. So, each drug was given
180 twice in the same week and we employed a mixed effects model to deal with any issues of inter-
181 class correlations and pseudoreplication.

182 Fixed effects were evaluated initially with compound symmetry assumed, and
183 subsequently with diagonal, first-order autoregressive (AR1) or unstructured covariance
184 structures. The best fitting model was ascertained by comparisons of Akaike's Information
185 Criterion (AIC). Denominator degrees of freedom were estimated according to the Satterthwaite
186 approximation. Data were analyzed in IBM[®] SPSS[®] Statistics (Version 21 for Macintosh). All
187 test statistics were evaluated with respect to an α -level of 0.05. All descriptive statistics are
188 reported as mean \pm standard error.

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Results

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[FIGURE 2 ABOUT HERE]

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Stability of baseline

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[FIGURE 3 ABOUT HERE]

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Figure 2 displays the learning curves during the first (1-sec FI PSI) and second (5-sec VI PSI) learning phases. As is clear, all fish increased their response accuracy during the course of the training, and this continued after the introduction of the 5-sec VI PSI. This was confirmed with a general LME comparing the first and second phase of learning (1-sec FI PSI vs 5-sec VI PSI), $F_{1,696} = 34.38, p < 0.001$. There was also a significant increase in anticipatory responses, $F_{1,724} = 588.01, p < 0.001$, and omissions, $F_{1,725} = 7.54, p < 0.01$, upon introduction of the 5-sec VI PSI. Finally, with respect to approach latency, there was no significant difference between the first and second phases of the experiment, $F_{1,439} = 2.79, p = 0.1$ (Phase 1 = 3.6 ± 1.42 secs vs. Phase 2 = 3.42 ± 1.11 secs), nor was there a difference for return latency, $F_{1,335} = 1.23, p = 0.27$ (Phase 1 = 12.61 ± 1.34 secs vs Phase 2 = 12.15 ± 0.93 secs).

Drug testing did not commence until the fish were performing ≥ 20 trials in a session. Prior to drug testing we examined performance over the final 5 sessions of pre-testing to ensure stability. A linear mixed effects model with day as the fixed effect revealed that accuracy had stabilized prior to drug testing commencing, $F_{4,83} = 1.95, p = 0.11$, as had anticipatory responding, $F < 1$. Omission errors, however, were not stable, $F_{4,83} = 4.97, p < 0.01$. Stability during the baseline days of drug training was confirmed for anticipatory responding, $F_{11,181} =$

211 1.14, $p = 0.33$. However accuracy, $F_{11,181} = 3.38$, $p < 0.01$ and omission errors, $F_{11,179} = 2.46$, $p <$
212 0.01 were variable during baseline (see Figure 3).

213

214 **Training**

215 [FIGURE 4 ABOUT HERE]

216 Figure 4 displays the number of trials, accuracy, anticipatory responding, omissions,
217 correct latency and return latency during the drug phase. There was a significant effect of drug
218 treatment on total number of trials completed (generalized LME with Poisson distribution), $F_{6,378} = 2.32$, $p < 0.05$. Post-hoc pairwise comparisons confirmed a dose-dependent change for
219 methylphenidate treatment. There was a significant increase in trials between baseline and 15 μ M
220 methylphenidate ($p = 0.041$), but no differences between baseline and 10 μ M ($p = 0.14$) or 5 μ M
221 methylphenidate ($p = 0.18$), nor between methylphenidate doses ($ps > 0.65$). There was no difference between
222 baseline and atomoxetine at any of the doses ($ps > 0.22$) nor between atomoxetine doses ($ps >$
223 0.89).

225 Drug treatment had no significant effect on proportion of correct responses during
226 sessions, $F < 1$. There was a significant main effect of drug treatment on anticipatory responses,
227 $F_{6,363} = 2.64$, $p < 0.05$. Pairwise comparison revealed that atomoxetine had a dose-dependent
228 effect. Specifically, 2 μ M atomoxetine reduced anticipatory responses relative to baseline ($p <$
229 0.01), but neither 1 μ M nor 0.5 μ M atomoxetine had any effect ($ps > 0.23$). There was no
230 difference between 2 μ M, 1 μ M or 0.5 μ M atomoxetine ($ps > 0.13$). Methylphenidate also affected
231 anticipatory responding, increasing it relative to baseline at 15 μ M ($p < 0.5$). There were no
232 differences at 5 μ M or 10 μ M compared to baseline ($ps > 0.25$). The fish also performed
233 significantly more anticipatory responses at 15 μ M methylphenidate than at 10 μ M ($p < 0.05$), but

234 no difference between 15 and 5 μ M ($p = 0.2$). There was no effect of drug treatment on
235 omissions, $F_{6,361} = 1.68$, $p = 0.12$, or approach latency, $F < 1$, or return latency, $F_{6,109} = 1.86$, p
236 = 0.09.

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Discussion

239 The aim of the present study was to carry out an initial pharmacological validation of a fully
240 automated version of the 5-CSRTT for studying impulse control in zebrafish. We previously
241 demonstrated that a low dose of amphetamine (0.025 mg/kg) reduced anticipatory responding
242 relative to saline injection on a 3-choice version of this task (Parker et al. 2012a). Here, we show
243 that atomoxetine reduced anticipatory responding in a dose-dependent manner (2 μ M
244 [0.6mg/Kg]), and methylphenidate increased anticipatory responding at higher doses (15 μ M
245 [4mg/Kg]). Methylphenidate also increased the number of trials completed during training
246 sessions, suggesting increased general activity levels following exposure to higher doses of this
247 drug. Neither compound had an effect on performance accuracy or omissions, nor any aspect of
248 response latency at the doses tested here. However, performance of zebrafish was variable during
249 baseline in terms of omission errors and to a lesser extent, accuracy, suggesting that the present
250 manifestation of this task may not be suitable for addressing attentional performance. Our data
251 show that in fish, selective increases in noradrenergic activity increase the ability to withhold a
252 response on this task representing similar patterns to those observed in rats (Robinson et al.
253 2008) and human patients with ADHD (Chamberlain et al. 2007). This suggests some degree of
254 conservation of the neurobiological underpinnings of the ability to withhold a response across
255 species.

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256 We also observed a higher proportion of anticipatory responses following incubation in
257 the maximum dose of methylphenidate (15 μ M), and intensification of general activity at all
258 doses, the latter as evidenced by the significant increase in completed trials in a session. The
259 increase in anticipatory responding and the increase in general activity levels are similar to those
260 observed in rats following comparably high doses of methylphenidate (5 mg/kg; Navarra et al.
261 2008) and amphetamine (Cole and Robbins 1987; 1989). Methylphenidate blocks both the
262 norepinephrine and dopamine transporter, thus causing a general increase in catecholamine
263 neurotransmission (Bymaster et al. 2002). The fact that methylphenidate did not reduce
264 anticipatory responding in the fish at the lower doses used here may suggest that the doses used
265 here may not have been appropriate for this species. This hypothesis is partially supported by the
266 fact that in a previous study we found that a very low dose of amphetamine (0.025mg/Kg), a
267 similar catecholaminergic transporter blocker, reduced anticipatory responding relative to saline
268 injection (Parker et al. 2012a). However, we based the doses here on previous work with larval
269 zebrafish (Lange et al. 2012) as well as effective doses used in mammalian models (Bizarro et al.
270 2004). In addition, the effect of methylphenidate on anticipatory responses on the 5-CSRTT are
271 highly variable, with some studies finding increases (Milstein et al. 2010; Navarra et al. 2008),
272 some no effect (Fernando et al. 2012) and some decreases (Bizarro et al. 2004) even at
273 comparable doses to one another (2.5-10mg/kg).

274 Zebrafish share a large degree of homology with mammals with respect to
275 catecholaminergic and monoaminergic neurotransmitter systems (Parker et al. 2013a).
276 Functional homologues for midbrain regions related to impulsivity are present in zebrafish, such
277 as the caudal raphe complex (Rink and Wullimann 2002), from which serotonergic (5-HT)
278 neurons project to the dorsal pallium (fish) and pre-frontal regions (mammals). It is also clear

279 that both dopamine (DA) and 5-HT projections from the pallium to thalamic regions are very
280 similar to those seen in mammals (Guo et al. 1999; Holzschuh et al. 2001; Rink and Guo 2004;
281 Rink and Wullimann 2001; 2002). Of relevance to this study, zebrafish have strikingly similar
282 projection patterns of catecholaminergic neurons; for example, norepinephrine neural projections
283 from the locus coeruleus to the subpallium in zebrafish and to the cortex in mammals (Holzschuh
284 et al. 2001; Korf et al. 1973; Ma 1997; Tay et al. 2011). The currently accepted hypothesis is that
285 the route of action of both atomoxetine and methylphenidate is via the reduction of locus
286 coeruleus activity (Pliszka et al. 1996). In addition, atomoxetine (1 μ M) and methylphenidate
287 (10 μ M) rescued the hyperactive/motor-impulsive phenotype observed in a putative ADHD
288 model using morpholino oligonucleotide-treated zebrafish larvae with a transient loss of function
289 in the latrophilin 3 (lphn-3) gene (Lange et al. 2012). This, in conjunction with our findings that
290 adult zebrafish respond similarly to atomoxetine in terms of anticipatory responding on a 5-
291 CSRTT to mammalian models and humans, suggest that this species may represent a useful
292 model system for examining the cellular and molecular basis of psychiatric disorder linked to
293 impulse control and for first round drug screening.

294 There are a number of performance, task-related and methodological differences between
295 fish and mammals on this task that should be addressed here. First, the proportion of correct
296 responses is lower in fish (~60% at asymptote) than rodents (~80-90% at asymptote) and the
297 response and return latencies are much longer in fish (~5 sec in fish vs. ~1 sec in rodents). In
298 addition, stability of baseline responding in terms of accuracy and omission errors appears to be
299 difficult to attain in fish. It may be that further refinement of the procedure will improve this in
300 the future, or it may reflect specific differences in task-performance between the species. For
301 example, fish may become satiated faster than rodents owing to their size and the amount of food

302 deliverable in each trial. If this were the case we may expect the rate of omission errors to be
303 correlated with accuracy, which we did not observe when all baseline sessions were considered.
304 However, in the final three baseline sessions, where accuracy increases (see Fig. 3a), omissions
305 increased in a similar manner consistent with the satiety hypothesis. Previously, we found
306 omission and accuracy to be correlated (Parker et al. 2012a). Alternatively, it may be that fish do
307 not stay on-task in the same way as rodents, meaning that they may not be capable of sustaining
308 attention for prolonged periods. This would result in lower reliability for accuracy and omission
309 errors, but will not necessarily affect premature responding as this aspect of performance would
310 be related to trial-specific motivation to approach the stimulus aperture.

311 There is some evidence that fish have differences in cognitive capacity; for example a
312 number of studies in the 1960s suggested that fish did not form attentional sets (Behrend et al.
313 1965; Bitterman 1965; Bitterman and Mackintosh 1969). However, this has since been shown to
314 be have been the result of poorly defined task-parameters (Parker et al. 2012b; Woodward et al.
315 1971). Second, the duration of the stimuli are shorter in the rodent version (~0.5-sec) than in fish
316 (30-sec) (Bari et al. 2008). We are unable to test fish at shorter stimulus durations, in particular
317 because zebrafish will become very stressed and not perform if confined to small areas. As such,
318 our testing tank is far larger in size relative to the size of the fish than the rodent assay. Therefore
319 we are not claiming that this task will be suitable for measuring aspects of attention in the fish
320 under the current protocol, but we hope that in the future, this might be incorporated into the
321 assay. Finally, in our design we incorporate a start gate in the apparatus. In the classical design of
322 the 5-CSRTT, the animal is required to perform a nose-poke the magazine and turn around to
323 start a trial. In our version, the fish has to return to the start area in order to drop the gate, and
324 subsequently re-start the task. In this sense, both versions rely on the animal performing an

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325 observing-response in order to gain access to the task stimuli. We have found that what appear to
326 be pre-potent responses in the fish can be induced by a variable interval pre-stimulus delay.
327 Furthermore we can, to some extent, control this with a noradrenaline transporter blocker
328 (atomoxetine), in a similar manner to that consistently observed in rodents. We would argue
329 therefore that this study represents a useful starting point for future research.

330 Zebrafish offer a valuable model for studying the genetics and molecular basis of
331 psychiatric disease in general (Guo 2004). There are numerous ethical and practical difficulties
332 relating to GWAS and CNV studies in humans, including an inability to test cause/effect
333 relations. This has led to the extensive use of animal models, often examining phenotypes
334 retrospectively using reverse-genetic procedures such as knock out/knock down of candidate
335 genes in murine models. Forward genetic screening procedures that use mutagenesis to introduce
336 random variation into the genome complement these studies and can uncover novel alleles and
337 pathways contributing to specific disease phenotypes (Muto et al. 2005). Mutagenesis studies in
338 rodents have been limited by both ethical and practical considerations, not least of which is the
339 small number of offspring in each generation (rodents have 5-10 offspring per pairing in
340 comparison to the 200-300 obtained from fish) and because levels of chemical mutagens
341 required to induce the high density of mutations per genome seen in zebrafish (1/300kb) are not
342 tolerated by rodents. In contrast, mutagenesis screening in zebrafish has been used to great effect
343 to uncover genetic modifiers of developmental processes (Amsterdam et al. 1999; Amsterdam et
344 al. 2004; Darland and Dowling 2001; Golling et al. 2002). The data we have described here
345 allow for behavioural screening in adult zebrafish to identify genetic modifiers of impulse
346 control.

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347 In summary, we have demonstrated that wild-type adult zebrafish show reduced
348 anticipatory responding on the 5-CSRTT with a comparable dose of atomoxetine (2 μ M) to those
349 observed in mammals. Taken with previous data from our lab (Parker et al. 2012a) and from
350 larval models of ADHD (Lange et al. 2012), this highly tractable and useful system, zebrafish, is
351 emerging as a potentially useful model for studying the cellular basis of impulsivity and for first-
352 round drug screening.

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506 Figure legends:

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509 *Figure 1.* Testing environment used to train zebrafish on 5-CSRTT. a) Gate mechanism,
 510 controlled by pneumatic piston. The gate raised to reveal the stimulus area containing the
 511 stimulus apertures, b), and the food delivery area containing the food magazine, c). The correct
 512 stimulus aperture, b), was signalled by illuminating a super-bright yellow LED, and food
 513 availability was signalled in the food magazine, c), by illuminating a super-bright green LED.
 514 Food, liquidized bloodworm and brine shrimp, was delivered via a 2ml plastic syringe, e), driven
 515 by a linear stepper motor, d), all mounted on an acylic base. Image detection was carried out
 516 using custom software (Python) and an HD webcam from above the tanks. (*Figure reproduced,*
 517 *with permission, from Parker et al., 2013b).*

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519

520 *Figure 2.* Training data from Phase 1 (1-sec FI PSI) and Phase 2 (5-sec VI PSI) of 5-CSRTT.
 521 Criterion for moving from Phase 1 to Phase 2 was ≥ 20 trials per session for three consecutive
 522 sessions. A) Correct responses increased steadily throughout training, and significantly increased
 523 between phases 1 and 2. B) Anticipatory responses increased on initiation of the 5-sec VI PSI. C)
 524 Omission errors increased significantly in phase 2. D) Summary of data in each training phase.
 525 Error bars represent SEM. *Note:* ** $p < 0.01$, post-hoc pairwise comparisons.

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527

528 *Figure 3.* Performance stability during baseline sessions of drug-trials. There was variability in
 529 accuracy (A), with accuracy increasing significantly in the last three days of baseline (days 58-
 530 61). There was also variability in omission errors (C), with omission error decreasing during
 531 days 51-58 of the drug delivery period, but re-stabilizing thereafter. Anticipatory response rate
 532 (B) was stable throughout the drug period. Error bars represent SEM. *Note:* Differs from Day 41
 533 * $p < 0.05$; ** $p < 0.01$, post-hoc pairwise comparisons.

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535

536 *Figure 4.* Dose-related effects of atomoxetine and methylphenidate on performance parameters
 537 of zebrafish in 5-CSRTT. A) Total trials in a session increased significantly (compared to
 538 baseline) following 15 μ M methylphenidate, but not at any other dose of either drug; B)
 539 Accuracy (proportion of correct responses) was not affected by either drug; C) Proportion of
 540 anticipatory responses was reduced (relative to baseline) following exposure to 2 μ M
 541 atomoxetine and increased following exposure to 15 μ M methylphenidate ; D) Proportion of
 542 omission errors was not affected by either drug; E) Approach latency and F) return latency to
 543 collect food were also unaffected by either drug. Error bars represent SEM. *Note:* Differs
 544 significantly from baseline * $p < 0.05$; ** $p < 0.01$, post-hoc pairwise comparisons.