Modafinil decreases anxiety-like behaviour in zebrafish

Adrian Johnson [1] , **Trevor James Hamilton** Corresp. [1, 2]

[1] Psychology, MacEwan University, Edmonton, Alberta, Canada

[2] Neuroscience and Mental Health Institute, Universtiy of Alberta, Edmonton, Canada

Corresponding Author: Trevor James Hamilton
Email address: trevorjameshamilton@gmail.com

Modafinil (2-((diphenylmethyl)sulfinyl)acetamide), a selective dopamine and norepinephrine transporter inhibitor, is most commonly prescribed for narcolepsy but has gained recent interest for treating a variety of disorders. Zebrafish (*Danio rerio)* are becoming a model of choice for pharmacological and behavioural research. To investigate the behavioural effects of modafinil on boldness, we administered doses of 0, 2, 20, and 200 mg/L for 30 minutes then tested zebrafish in the novel approach test. In this test the fish was placed into a circular arena with a novel object in the center and motion-tracking software was used to quantify the time the fish spent in the outer area of the arena (thigmotaxis zone), middle third of the arena (transition zone) and center of the arena, as well as total distance traveled, immobility and meandering. Modafinil caused a decrease in time spent in the thigmotaxis zone and increased time spent in the transition zone across all doses. Modafinil did not significantly alter the time spent in the center zone (near the novel object), the distance moved, meandering, or the duration of time spent immobile. These results suggest that modafinil decreases anxiety-like behaviour in zebrafish.

Modafinil decreases anxiety-like behaviour in zebrafish

Adrian Johnson[1], Trevor James Hamilton[1,2]*,

[1]Department of Psychology, MacEwan University, Edmonton, AB, Canada, T5J 4S2.

[2]Neuroscience and Mental Health Institute, University of Alberta, Edmonton, AB, Canada, T6G 2H7.

* Corresponding author: trevorjameshamilton@gmail.com, phone: 1-780-633-3584

Abstract

Modafinil (2-((diphenylmethyl)sulfinyl)acetamide), a selective dopamine and norepinephrine transporter inhibitor, is most commonly prescribed for narcolepsy but has gained recent interest for treating a variety of disorders. Zebrafish (*Danio rerio)* are becoming a model of choice for pharmacological and behavioural research. To investigate the behavioural effects of modafinil on boldness, we administered doses of 0, 2, 20, and 200 mg/L for 30 minutes then tested zebrafish in the novel approach test. In this test the fish was placed into a circular arena with a novel object in the center and motion-tracking software was used to quantify the time the fish spent in the outer area of the arena (thigmotaxis zone), middle third of the arena (transition zone) and center of the arena, as well as total distance traveled, immobility and meandering. Modafinil caused a decrease in time spent in the thigmotaxis zone and increased time spent in the transition

zone across all doses. Modafinil did not significantly alter the time spent in the center zone (near the novel object), the distance moved, meandering, or the duration of time spent immobile. These results suggest that modafinil decreases anxiety-like behaviour in zebrafish.

25

26 **Introduction**

27 Modafinil (2-((diphenylmethyl)sulfinyl)acetamide) (brand name Alertec in Canada, Provigil in
28 the United States, and Modavigil in Australia) is a psychostimulant primarily used by narcolepsy
29 patients and shift workers to alleviate sleep related disorders. It is being tested as a potential
30 treatment for major depressive disorder (MDD), cocaine-addiction, and as a cognitive enhancer
31 (Abolfazli, et al., 2011; Dean, et al., 2011; Minzenberg & Carter, 2008). Modafinil is thought to
32 inhibit dopamine transporters (Madras et al., 2006; Volkow et al., 2009) as well as and
33 norepinephrine transporters (Madras et al., 2006) leading to increased synaptic dopamine and
34 norepinephrine, respectively, but not to a level stimulating abuse of the drug. It is shown to
35 reduce GABA (y-aminobutyric acid) levels, and increase levels of serotonin (5HT), glutamate,
36 orexin, and histamines in the brain (Minzenberg & Carter, 2008, Mereu et al., 2013). Because of
37 its action on these neurotransmitter systems and low abuse profile, modafinil has the potential for
38 a wide therapeutic benefit.

39 Modafinil has a variety of effects on anxiety in humans and anxiety-like behaviour in
40 animal models. Some studies have shown anxiety generating (anxiogenic) effects in humans,
41 while others show anxiety reducing (anxiolytic) effects. In an emotion and cognition test, Rasetti,
42 et al. (2010) found that 7 days of modafinil (100 mg/day) increased the efficacy of prefrontal
43 cognitive information processing in humans, while reducing the reactivity to fearful and
44 threatening stimuli through the amygdala (controlling anxiety). However, in other studies,
45 repeated doses of modafinil given to sleep apnea patients (200-400 mg/day, 4 weeks; Schwartz,
46 Hirshkowitz, & Erman, 2003), and narcoleptic patients (400 mg/day, 2 weeks; Broughton, et al.,
47 1997) caused an increase in nervousness and/or anxiety. In another study, Randall, Shneerson,

Plaha, & File (2003) found that doses of 100-800 mg in 'healthy young volunteers' had significant anxiogenic effects. Therefore, it is still inconclusive as to what type of anxiety-altering response modafinil might produce in humans.

Often we can move to animal models to find answers to pharmacological questions, however, animal models have also shown differing effects of modafinil. In rhesus macaque monkeys (*Macaca mulatta*), single doses of modafinil have been shown to increase nocturnal activity but did not decrease anxiety responses (Hermant, et al., 1991). Van Vliet, Jongsma, Vanwersch, Olivier, & Philippens (2006) found an anxiolytic response in marmoset monkeys (*Callithrix jacchus*) when recording their startle responses to a threat situation after a single oral dose of modafinil (between 50-225 mg/kg). In male swiss albino mice (*Mus musculus*), Simon, Panissaud & Costentin (1994) compared modafinil to dexamphetamine (another known stimulant) and measured the relative stimulant properties as well as anxiety levels generated by these substances. Of the three tests used (black and white compartment test, elevated plus maze, and open field task), modafinil did not increase any anxiety behaviours. Interestingly, modafinil dose-dependently increases spontaneous exploration in C57BL/6J mice (Young et al., 2011). These studies in animal models also demonstrate an inconsistent effect of modafinil on anxiety and exploratory behaviour.

Zebrafish (*Danio rerio*), have become a popular animal model used in the scientific community due to their fecundity, larval-stage transparency, short gestational period, and ease in handling (Gerlai, 2010; Stewart et al., 2014). The similarity in zebrafish genetic and behavioural markers to those in humans, make this species-an ideal model organism for studying pharmacological compounds (Spence et al., 2008; Tierney 2011). To date only one study examined the effect of modafinil on zebrafish behaviour and it was performed on larval zebrafish

(6 days post-fertilization). Sigurgeirsson et al., (2011) found that modafinil causes a dose-dependent reduction in sleep. Specifically, they found that modafinil had no effect on the number of transitions between sleep and wakefulness throughout a 24 hour monitoring period, but had a decrease in the mean sleep percentages of fish as the dosages increased, especially in the 12 hours in which the lights were off at night. Modafinil increased the wakeful bouts of fish whilst maintaining the structure of sleep, however these researchers did not perform any cognitive or anxiety-related behavioural tests. To date, the effects of modafinil in larval or adult zebrafish remains relativity unexplored.

In this study we used the novel approach test, a well-validated test of anxiety in zebrafish (Stewart et al., 2012) and other fish species (Ou et al., 2015). In this test the fish is placed into a circular arena with a novel object in the center. Typically, zebrafish are fearful of the novel object (neophobic) and spend time near the wall of the arena (thigmotaxis). With a camera-based motion-tracking software system we quantified the time near the object, near the wall, and in a transition zone in the middle, as well as distance moved, meandering, and immobility, after a 30 minute exposure to modafinil (2, 20, and 200 mg/L).

Materials and Methods

The experimental procedures were approved by the MacEwan University Animal Research Ethics Board (AREB; protocol number 05-12-13). These standards are in compliance with the Canadian Council for Animal Care (CCAC).

Subjects

This study used 100 adult zebrafish (*Danio rerio)* that were held in a 3-shelf benchtop housing system (Aquatic Habitats) in either 3 or 10 L polypropylene tanks. The fish were

obtained from Aquatic Imports (Calgary) and the gender was unidentified. Habitat water was made in the lab by purifying tap water through reverse osmosis (RO), then adding 5 mL of prime (sodium thiosulphate), 25 g of aquarium salt, 10-18 g of $NaHCO_3$, and 100 mL of acetic acid were added for every 60 L of RO water. The water in the habitat was continuously recirculated, treated with activated carbon, and was UV irradiated and filtered. The zebrafish were fed once daily on an alternating diet of either flake food (Gamma-micro 300, New Life Products) or shrimp (Omega One Freeze Dried Shrimp Nutri-Treat, Omega Sea Ltd.). Fish were kept on a daily 12-hour light/dark cycle with lights turning on at 8AM and off at 8PM. The water conditions in the habitat were monitored daily with the temperature maintained between 26.0 ^oC and 29.0 ^oC, the pH between 7.0 and 8.0, and the dissolved O_2 levels between 5.0 and 10.0 ppm.

Drug Administration

Modafinil (Toronto Research Chemicals) was administered to the fish at three different concentrations; 2 (n=19), 20 (n=23), or 200 (n=22) mg/L, by first dissolving powdered modafinil in dimethyl sulfoxide (DMSO) to increase solubility. This DMSO/modafinil solution was mixed with 250 mL of habitat water to achieve the desired concentration. The control group (n=35) underwent otherwise identical experimental procedures (DMSO added to 250 mL of habitat water) without the presence of modafinil. Zebrafish were individually administered modafinil or control solutions for 30 minutes before testing began. Each drug group was performed on two days of testing, with controls interspersed throughout the day at a 2:1 (drug:control) ratio, resulting in ~n=20 for each drug group and n=35 for the controls. The drug solution was made fresh each day and maintained at 26-28 ^oC. All of the fish used in this experiment were experimentally and drug naïve.

Behavioural Testing

116 Testing was performed during the light hours of 12-hour light/dark cycle. On the day of
117 testing, the fish were transported in their housing tank to the experimental area. The fish were
118 individually placed in either the control or modafinil solution (2, 20, or 200 mg/L) for 30
119 minutes. Following drug administration the fish were placed in the circular arena placed into the
120 novel approach test arena; a circular white plastic arena with at diameter of 34 cm and walls that
121 were 16 cm high (Figure 1A). The arena was filled with 6 cm of habitat water that was
122 maintained at 26-28 ^{o}C using a heat mat (Seedling Heat Map – HydroFarm) placed underneath
123 the arena. A Lego figurine was placed in the middle of the arena during testing and was used as
124 the novel object in this paradigm (Figure 1B) similar to other studies (Ou et al., 2015; Kwan et
125 al., submitted). It was held in place with a 1.5 cm x 1.5 cm square of Velcro glued to the feet of
126 the Lego figurine and the arena floor. The height of the water completely submerged the
127 figurine. The fish were placed into the arena and recorded for 10 minutes. Dependent variables
128 were measured using EthoVision XT (version 10; Noldus) motion tracking software. In
129 EthoVision, the arena was divided into three zones, the center, transition, and thigmotaxis zone.
130 The center zone consisted of a 10 cm diameter circle with the middle on top of the object. The
131 thigmotaxis zone was a circular zone from the wall 4.5 cm (one body length of a zebrafish)
132 toward the center of the arena. The transition zone was the zone in between the center zone and
133 thigmotaxis zone. The variables measured were time in each zone (center, transition,
134 thigmotaxis), distance moved, meandering, and duration immobile. Following the behavioural
135 testing the fish were placed back into their housing tanks.

136 **Statistical Analysis**

137 A D'Agostino & Pearson omnibus normality test was used to assess normality for all data
138 sets. One-way ANOVA with a post hoc Dunn's Multiple Comparison Test was used with

parametric data and nonaprametic data was analyzed with a Kruskal-Wallis test. Absolute time in each zone was measured as duration (s) in zones for the full 10 minute trial. Meandering was quantified by dividing the turn angle of each fish by the distance moved and calculated in Ethovision. Immobility was defined in as the percent change in the pixels of the fish from frame to frame and was set at a threshold of 5% (Pham et al., 2009). The behavioural data was analyzed using GraphPad Prism software (version 6). Data are shown as mean ± s.e.m.

Results

Zebrafish were exposed to doses of 0 (control), 2, 20, and 200 mg/L for 30 minutes then immediately tested zebrafish in the novel approach test. Figure 2 is a representative example of the movement of one zebrafish over the duration of the 10 minute trial under control conditions (Figure 2A) and another zebrafish after being exposed to 200 mg/L of modafinil (Figure 2B). The heatmaps are examples of the same responses but with pseudocolour representation of the location of the fish over time (Figures 2C-D).

Time in zones

To examine the effects of modafinil on anxiety and exploratory behaviour using the novel approach test, the time spent in the center, transition, and thigmotaxis zones were measured. We found that time spent in the thigmotaxis zone was significantly increased for all modafinil groups compared to control (Figure 3A; control: 494.2 ± 9.8 s, 2 mg/L: 449.3 ± 15.8 s, 20 mg/L: 441.4 ± 12.5 s, 200 mg/L: 448.1 ± 16.5 s; $F(3,96) = 4.168$, $P = 0.008$). Time spent in the transition zone was also significantly greater for all groups compared to controls (Figure 3B, control: 101.5 ± 9.3 s, 2 mg/L: 144.9 ± 14.7 s, 20 mg/L: 149.9 ± 11.9 s, 200 mg/L: 144.4 ± 15.1s; $F(3,96) = 4.136$, $P = 0.008$). Although there was a trend towards a dose-dependent effect of modafinil on

time spent in the center zone, there was no significant difference in time spent in the center across all groups (Figure 3C; control: 4.3 ± 0.9s, 2 mg/L: 5.8 ±1.5 s, 20 mg/L: 8.7 ± 2.5 s, 200 mg/L: 7.5 ± 1.7s; H (3) = 5.727, P = 0.1257).

Locomotion

To examine the locomotor activity of fish dosed with modafinil we quantified the distance moved, meandering, and duration the fish was immobile. The drug treatment groups did not significantly differ from the control group in the distance moved (Figure 4A; control 5537 ± 164 cm, 2 mg/L: 5957 ± 328 cm, 20 mg/L: 5410 ± 266 cm, 200 mg/L: 5288 ± 209 cm, F(3, 96) = 1.277, p = 0.2868). No significant difference was found for meandering (Figure 4B; control: 158.8 ± 62.9 °/cm, 2 mg/L: 115.6 ± 74.3 °/cm, 20 mg/L: 339.1 ± 159.0 °/cm, 200 mg/L: 814.9 ± 438.0 °/cm, H (3) = 5.376, P = 0.1463) across all groups. There was no significant difference in immobility (Figure 4C: control: 4.9 ± 1.6 s, 2 mg/L: 3.8 ± 2.2 s, 20 mg/L: 11.8 ± 5.2 s, 200 mg/L: 17.3 ± 7.9 s, H (3) = 4.032, P = 0.2580) across all groups.

Discussion

Our findings show that the acute administration of modafinil decreases anxiety-like behaviour in zebrafish across a range of doses. In particular, zebrafish administered 2, 20, and 200 mg/L modafinil spent significantly less time in the thigmotaxic zone near the walls of the arena, and more time in the transition zone of the novel approach test (Figures 2 and 3). This type of behaviour is consistent with decreased anxiety, and has been observed in zebrafish with anxiolytic compounds resulting in less time near the walls of an open field test (Maximino et al., 2010). We observed no significant changes in locomotion; distance moved, meandering, and

183 immobility, therefore, their zone preference was not due to a locomotor alteration. These results
184 suggest that modafinil decreases anxiety in zebrafish.

185 The novel approach test involves placing a fish into a circular arena containing a novel
186 object in the center. The innate fear response for a never-before seen object (neophobia) is the
187 behaviour of interest in zebrafish, as they have a tendency to remain away from the object that
188 they may perceive as a predator (Wright et al., 2006, Moretz et al., 2007). This has been
189 pharmacologically validated in juvenile pink salmon with the $GABA_A$ receptor antagonist,
190 gabazine, an anxiogenic compound that decreased time near the novel object and increased time
191 in the thigmotaxis zone (Ou et al., 2015). In the present study all doses of modafinil decreased
192 time in the thigmotaxis zone, suggesting that anxiety is reduced. There was a trend towards a
193 decrease in distance moved and increase in meandering as dosage increased, suggesting a
194 potential dose-dependent effect of modafinil on activity, however this did not reach significance.
195 These findings are consistent with a modafinil-induced increase in rearing and holepoking in
196 mice, which is considered exploratory behaviour, but somewhat in contrast with the elevated
197 hyperactivity observed in mice (Young et al., 2011).

198 Modafinil has been shown to occupy bindings sites on the dopamine transporter (DAT)
199 and norepinephrine transporter (NAT) with modest affinity compared to other psychostimulants
200 like methylphenidate (Ritalin; Madras et al., 2006; Minzenberg and Carter, 2008). This action
201 on the catecholamine system has been shown to increase extracellular dopamine in the medial
202 prefrontal cortex of rats (de Saint Hilaire et al., 2001), striatum of mice (Wisor et al., 2001) and
203 nucleus accumbens of rats (Ferraro et al., 1997; Munillo-Rodriuquez et al., 2007). In a recent
204 study on the effects of modafinil on dopamine receptor knockout mice the exploratory effect of
205 modafinil was attenuated in drd1 and drd4 knockout mice, supporting the role of the dopamine

D1 and D4 receptors in the increased exploratory response to elevated synaptic dopamine caused by modafinil (Young et al., 2011). Extracellular norepinephrine has also been shown to be increased in the prefrontal cortex and hypothalamus and extracellular serotonin increased in the prefrontal cortex and hypothalamus (de Saint Hilaire et al., 2001) frontal cortex and amygdala (Ferraro et al., 2000, 2002) of rats. In the current study we did not perform intracranial microdialysis or use receptor knockouts, however, it is likely that elevated extracellular dopamine, norepinephrine, and serotonin are also at least in part responsible for the anxiolytic effects seen in zebrafish. In other zebrafish research there is ample evidence that modulation of monoamine neurotransmission leads to changes in exploratory behaviour and anxiety. Using the norepinephrine reuptake inhibitor, desipramine (25 mg/L), and the serotonin reuptake inhibitor, citalopram (100 mg/L), Sackerman et al., (2010) demonstrated that each of these compounds increased time spent in the upper region of a novel tank diving test which is indicative of decreased anxiety. Also using the novel tank diving test, Bencan and colleagues (2009) found that buspirone (6.25 – 50 mg/L) which is mainly a serotonin 1A agonist (with low affinity to the dopamine D2 receptor agonist where it acts as an antagonist, and weak affinity to serotonin 2 receptor) (Loane & Politis, 2012) also had an anxiolytic effect. Chronic administration of fluoxetine (100 μg/L for 2 weeks), a selective serotonin reuptake inhibitor also produced a significant increase in time spent in the top of a novel tank diving test, decreasing anxiety. Future research with genetic knockouts in zebrafish, like the *per1b* which expresses low levels of dopamine (Huang et al., 2015) would be ideal to explore the mechanism of action of modafinil in

The novel approach test has been considered an appropriate test to assess anxiety in zebrafish (Wright et al., 2006, Stewart et al., 2012), although, to the best of our knowledge, has never been used with pharmacological compounds in zebrafish (Maximino et al., 2010). This

research supports the novel approach test as a legitimate test for anxiety and exploratory behaviour in zebrafish and could be used with a variety of compounds. The present findings suggest that modafinil has anxiolytic properties due to the decrease in time the fish spent in the thigmotaxis zone and the increase in time the fish spent in the transition zone. Future research should test the potential nootropic properties of modafinil on zebrafish using learning paradigms like the novel object recognition test (May et al., 2015), episodic-like memory test (Hamilton et al., 2016), food-based reinforcement tasks (Ingraham et al., 2016), or other appetitive conditioning, associative learning or aversive conditioning learning and memory tests (see Gerlai, 2016 for review). If successful at enhancing memory formation or storage, modafinil could then be administered with a putative model of Alzheimer's disease, the prion protein knockout model (Leighton et al., *under review*), in an attempt to rescue these deficits. Once the complex drug profile of modafinil is understood, clinicians may be able to adequately prescribe it to a wide range of patients.

Figure Legends

Figure 1. Apparatus used in the novel approach test. (A) The arena used was a plastic circular arena (diameter: 34 cm, wall height: 16 cm) placed on a seeding mat to maintain water temperature. (B) The novel object was a multicolour Lego figurine (height: 5 cm, width: 1.5 cm).

Figure 2. Individual fish movement in the novel approach test. (A) Trackplot of an individual fish in the control condition for the 10 minute trial. (B) Trackplot of an individual fish in the 200 mg/L modafinil condition. (C) Heatmap of the same fish from the control condition in (A). A heatmap is a visualization of the location of the fish over the entire 10 minute trial. (D) Heatmap for the same fish from the modafinil 200 mg/L condition in (B).

Figure 3. Time in zones of the novel approach test. (A) The time spent in the thigmotaxis zone (near walls) of the arena decreased with modafinil. (B) The time spent in the transition zone (middle zone) of the arena increased with modafinil. (C) The time spent in the center zone (where the object was located) was not significantly different across groups. * $p < .05$, ** $p < 0.01$, difference from control group.

Figure 4. Locomotion variables in the novel approach test. (A) There was no significant difference in distance moved across all groups. (B) There was no significant difference in meandering across all groups. (C) There was no significant difference in immobility across all groups.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery grant to T.J.H.) grant number 04843. We would also like to thank Aleah McCory (Animal Care Technician), Melike Schalomon, Taylor Pitman, Anne Walley, and Erica Loh, for their assistance with fish husbandry. We are very grateful to Dr. Gregory de Pascale for his helpful comments on this manuscript and to Melissa Bryant for helping with the conception of this study.

References

Abolfazli, R., Hosseini, M., Ghanizadeh, A., Ghaleiha, A., Tabrizi, M., Raznahan, M., & Akhondzadeh, S. (2011). Double-blind randomized parallel-group clinical trial of efficacy of the combination fluoxetine plus modafinil versus fluoxetine plus placebo in the treatment of major depression. *Depression and Anxiety*, *28(4),* 297-302. doi:10.1002/da.20801.

Bencan, Z., Sledge, D., & Levin, E. D. (2009). Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *Pharmacology, Biochemistry and Behaviour, 94*, 75-80. doi: 10.1016/j.pbb.2009.07.009

Broughton, R. J., Fleming, J. A. E., George, C.F. P., Hill, J. D., Kryger, M. H., Moldofsky, H., Montplaisir, J. Y., Morehouse, R. L., Moscovitch, A., & Murphy, W. F. (1997). Randomized, double-blind, placebo-controlled crossover trial of modafinil in the treatment of excessive daytime sleepiness in narcolepsy. *Neurology*, 49(2), 444-451. doi:10.1212/WNL.49.2.444

Dean, A. C., Sevak, R. J., Monterosso, J. R., Hellemann, G., Sugar, C. A., & London, E. D. (2011). Acute modafinil effects on attention and inhibitory control in methamphetamine-dependent humans. *Journal Of Studies On Alcohol And Drugs*, 72(6), 943-953. doi: 10.15288/jsad.2011.72.943

de Saint Hilaire, Z., Orosco, M., Rouch, C., Blanc, G., Nicolaidis, S. (2001). Variations in extracellular monoamines in the prefrontal cortex and medial hypothalamus after modafinil administration:a microdialysis study in rats. *NeuroReport* 12: 3533–3537.

Ferraro, L., Antonelli, T., O'Connor, W.T., Tanganelli, S., Rambert, F.A., Fuxe, K. (1997) Modafinil: an antinarcoleptic drug with a different neurochemical profile to d-amphetamine and dopamine uptake blockers. *Biol Psychiatry*. 42:1181–3.

Ferraro, L., Fuxe, K., Tanganelli, S., Fernandez, M., Rambert, F.A., Antonelli, T. (2000) Amplification of cortical serotonin release: a further neurochemical action of the vigilance-promoting drug modafinil. *Neuropharmacology*. 39:1974–83.

Ferraro, L., Fuxe, K., Tanganelli, S., Tomasini, M.C., Rambert, F.A., Antonelli, T. (2002) Differential enhancement of dialysate serotonin levels in distinct brain regions of the awake rat by modafinil: possible relevance for wakefulness and depression. *J Neurosci Res*. 68:107–12.

Gerlai, R. (2010) High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. *Molecules*. 15(4): 2609-22. doi: 10.3390/molecules15042609.

Gerlai, R. (2016) Learning and memory in zebrafish (Danio rerio). *Methods Cell Biol*. 134: 551-86.

Hamilton, T.J., Myggland, A., Duperreault, E. May, Z., Gallup J., Powell, R.A., Schalomon, M., & Digweed, S. (2016). Episodic-like memory in zebrafish. *Anim Cogn,*

doi:10.1007/s10071-016-1014-1

Hermant, J. F., Rambert, F. A., Duteil, J. (1991). Awakening properties of modafinil: Effect on nocturnal activity in monkeys (*Macacamulatta*) after acute and repeated administration. *Psychopharmacology*; 103(1):28–32.

Huang, J., Zhong, Z., Wang, M., Chen, X., Tan, Y., Zhang, S., He, W., He, X., Huang, G., Lu, H.,Wu, P., Che, Y., Yan, Y., Postlethwait, J.H., Chen, W.,Wang, H. (2015). Circadian Modulation of Dopamine Levels and Dopaminergic Neuron Development Contributes to Attention Deficiency and Hyperactive Behavior. *J Neurosci.* 35(6): 2572–2587. doi: 10.1523/JNEUROSCI.2551-14.2015

Ingraham, E., Anderson, N.D., Hurd, P.L., & Hamilton T.J. (2016) Twelve-Day Reinforcement-Based Memory Retention in African cichlids (*Labidochromis caeruleus*). *Frontiers in Behavioural Neuroscience*. 10. doi:10.3389/fnbeh.2016.00157

Kwan G., Hamilton, T.J. Tresguerres M. (*submitted*). CO_2-induced ocean acidification does not affect individual or group behaviour in temperate damselfish (*Chromis punctipinnis*)

Lecendreux, M., Bruni, O., Franco, P., Gringras, P., Konofal, E., Nevsimalova, S., Paiva, T., Partinen, M., Peeters, E., Peraita-Adrados, R., Plazzi, G., & Poli, F. (2012). Clinical experience suggests that modafinil is an effective and safe treatment for paediatric narcolepsy. *Journal Of Sleep Research*, *21*(4), 481-483. doi:10.1111/j.1365-2869.2011.00991.x

Leighton, P.L.A., Nadolski, N., Morrill, A., Hamilton, T.J., & Allison, W.T. (*under review*). An ancient conserved role for prion protein in learning & memory. *PLoS ONE.*

Loane, C. & Politis M. (2012) Buspirone: what is it all about? *Brain Res.* 1461:111-8. doi: 10.1016/j.brainres.2012.04.032.

Madras, B.K., Xie, Z., Lin, Z., Jassen, A., Panas, H., Lynch, L. et al (2006). Modafinil occupies dopamine and norepinephrine transporters in vivo and modulates the transporters and trace amine activity in vitro. *J Pharmacol Exp Ther.* 319: 561–569.

Maximino, C., de Brito, T. M., da Silva Batista, A. W., Herculano, A. M., Morato, S., & Gouveia, A. (2010). Measuring anxiety in zebrafish: a critical review. *Behavioural brain research*, *214*(2), 157-171.

May, Z, Morrill, A, Holcombe, A, Johnston, T, Gallup, J, Fouad, K, Schalomon, M, Hamilton, T.J. (2015) Object recognition memory in zebrafish. *Behavioural Brain Research*. *296*, 199-210.

Mereu, M, Bonci, M., Newman, A.H., Tanda, G. (2013). The neurobiology of modafinil as an enhancer of cognitive performance and a potential treatment for substance use disorders. *Psychopharmacology (Berl)*. 229(3): 415–434. doi:10.1007/s00213-013-3232-4.

Minzenberg, M. J., & Carter, C. S. (2008). Modafinil: A review of neurochemical actions and effects on cognition. *Neuropsychopharmacology*, 33(7), 1477-1502. doi:10.1038/sj.npp.1301534

Moretz, J. A., Martins, E. P., & Robinson, B. D. (2007). Behavioral syndromes and the evolution of correlated behaviour in zebrafish. Behavioral Ecology, 18, 556-562. doi:10.1093/beheco/arm011

Murillo-Rodriguez, E., Haro, R., Palomero-Rivero, M., Millan-Aldaco, D., Drucker-Colin R. (2007). Modafinil enhances extracellular levels of dopamine in the nucleus accumbens and increases wakefulness in rats. *Behav Brain Res*. 176:353–7.

379 Ou, M., Hamilton, T. J., Eom, J., Lyall, E. M., Gallup, J., Jiang, A., Lee, J., Close, D. A., Yun, S.
380 S., & Brauner, C. J. (2015). Responses of pink salmon to CO2-induced aquatic
381 acidification. *Nature Climate Change*, *5*(10), 950-955.

382 Pham, J., Cabrera, S. M., Sanchis-Segura, C., & Wood, M. A. (2009). Automated scoring of
383 fear-related behavior using EthoVision software. *Journal Of Neuroscience Methods,*
384 178(2), 323-326. doi:10.1016/j.jneumeth.2008.12.021

385 Randall, D. C., Shneerson, J. M., Plaha, K. K., & File, S. E. (2003). Modafinil affects mood, but
386 not cognitive function, in healthy young volunteers. H*uman Psychopharmacology:*
387 *Clinical And Experimental,* 18(3), 163-173. doi:10.1002/hup.456

388 Rasetti, R., Mattay, V. S., Stankevich, B., Skjei, K., Blasi, G., Sambataro, F., & Weinberger, D.
389 R. (2010). Modulatory effects of modafinil on neural circuits regulating emotion and
390 cognition. *Neuropsychopharmacology,* 35(10), 2101-2109. doi:10.1038/npp.2010.83

391 Sackerman, J., Donegan, J. J., Cunningham, C. S., Nguyen, N. N., Lawless, K., Long,
392 A.,...Gould, G. G. (2010). Zebrafish behaviour in novel environments: Effects of acute
393 exposure to anxiolytic compounds and choice of Danio Rerio line. *International Journal*
394 *of Comparative Psychology, 23*(1), 43-61.

395 Schwartz, J. R., Hirshkowitz, M., Erman, M. K. (2003). Modafinil as adjunct therapy for daytime
396 sleepiness in obstructive sleep apnea: a 12-week, open-label study. 124(6):2192–2199.
397 [PubMed: 14665500])

398 Sigurgeirsson, B., Porsteinsson, H., Arnardottir, H., Johannesdottir, I. P., & Karlsson, K. E.
399 (2011). Effects of Modafinil on sleep-wake cycles in larval zebrafish, *Zebrafish,* 0, 1-8
400 doi: 10.1089/zeb.2011.0708

401 Simon, P., Panissaud, J., & Costentin, J. (1994). The stimulant effect of modafinil on
402 wakefulness is not associated with an increase in anxiety in mice. Psychopharmacology.
403 114, 597-600.

404 Spence R, Gerlach G, Lawrence C, Smith C (2008) The behaviour and ecology of the zebrafish,
405 Danio rerio. Biol Rev Camb Philos Soc 83: 13–34. doi: 10.1111/j.1469-
406 185X.2007.00030.x.

407 Spiller, H. A., Borys, D., Griffith, J. R., Klein-Schwartz, W., Aleguas, A., Sollee, D. & Sawyer,
408 T. S. (2009). Toxicity from modafinil ingestion. *Clinical Toxicology*, *47*(2), 153-156.

409 Stewart, A., Gaikwad, S., Kyzar, E., Green, J., Roth, A., & Kalueff, A. V. (2012). Modeling
410 anxiety using adult zebrafish: a conceptual review. Neuropharmacology, 62(1), 135-143.

411 Stewart, A.M., Braubach, O., Spitsbergen, J., Gerlai, R., Kalueff, A.V. (2014) Zebrafish models
412 for translational neuroscience research: from tank to bedside. *Trends Neurosci*.
413 37(5):264-78. doi: 10.1016/j.tins.2014.02.011.

414 Tierney, K.B. (2011) Behavioural assessments of neurotoxic effects and neurodegeneration in
415 zebrafish. Biochim Biophys Acta, 1812: 381–389. doi: 10.1016/j.bbadis.2010.10.011.

416 Van Vliet, S.A., Jongsma, M. J., Vanwersch, R. A., Olivier, B., & Philippens, I. H. (2006).
417 Behavioral effects of modafinil in marmoset monkeys. *Psychopharmacology*, *185*(4),
418 433-440.

419 Wisor, J.P., Nishino, S., Sora, I., Uhl, G.H., Mignot, E., Edgar, D.M. (2001). Dopaminergic role
420
421 in stimulant-induced wakefulness. *J Neurosci*. 21: 1787–1794.
422
423 Wright, D., Nakamichi, R., Krause, J., and Butlin, R.K. (2006) QTL Analysis of Behavioral and
424 Morphological Differentiation Between Wild and Laboratory Zebrafish (Danio rerio).

Behav. Genet. 36(2): 271-284.

Young J.W., Kooistra K., Geyer M.A. (2011). Dopamine receptor mediation of the exploratory/hyperactivity effects of modafinil. *Neuropsychopharmacology* 36: 1358–1396

Figure 1(on next page)

All figures

Figure 1

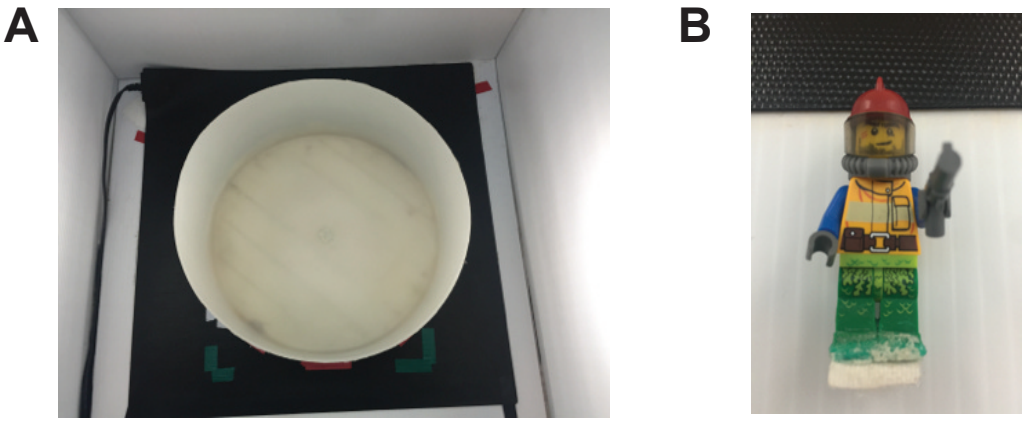


Figure 2

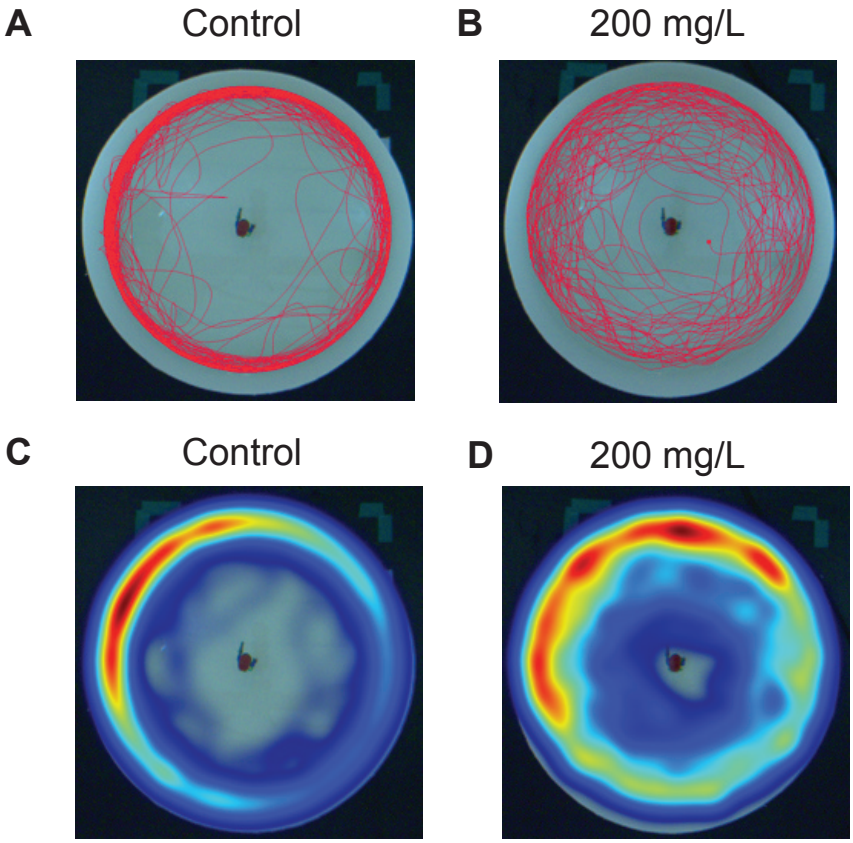


Figure 3

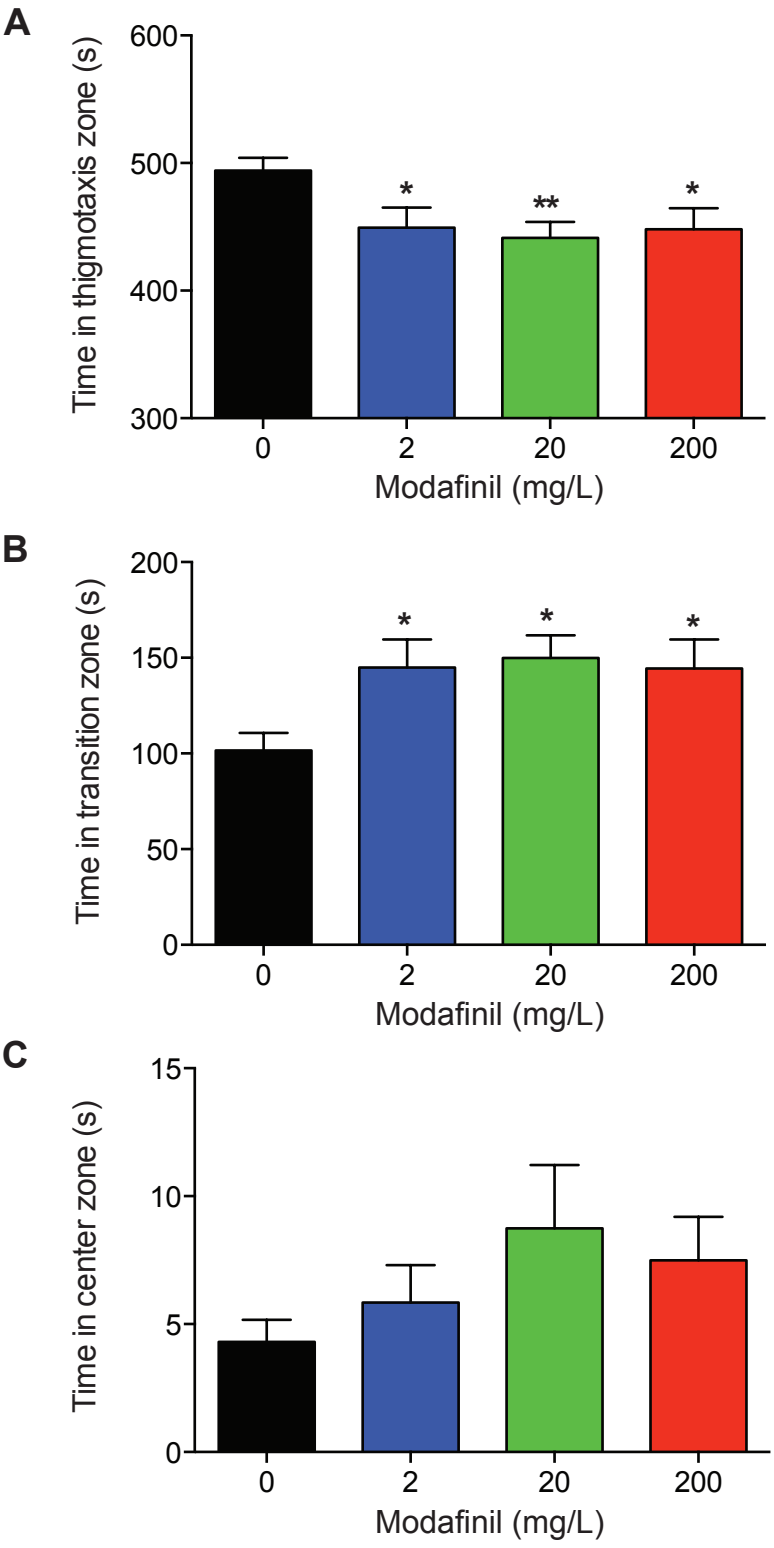
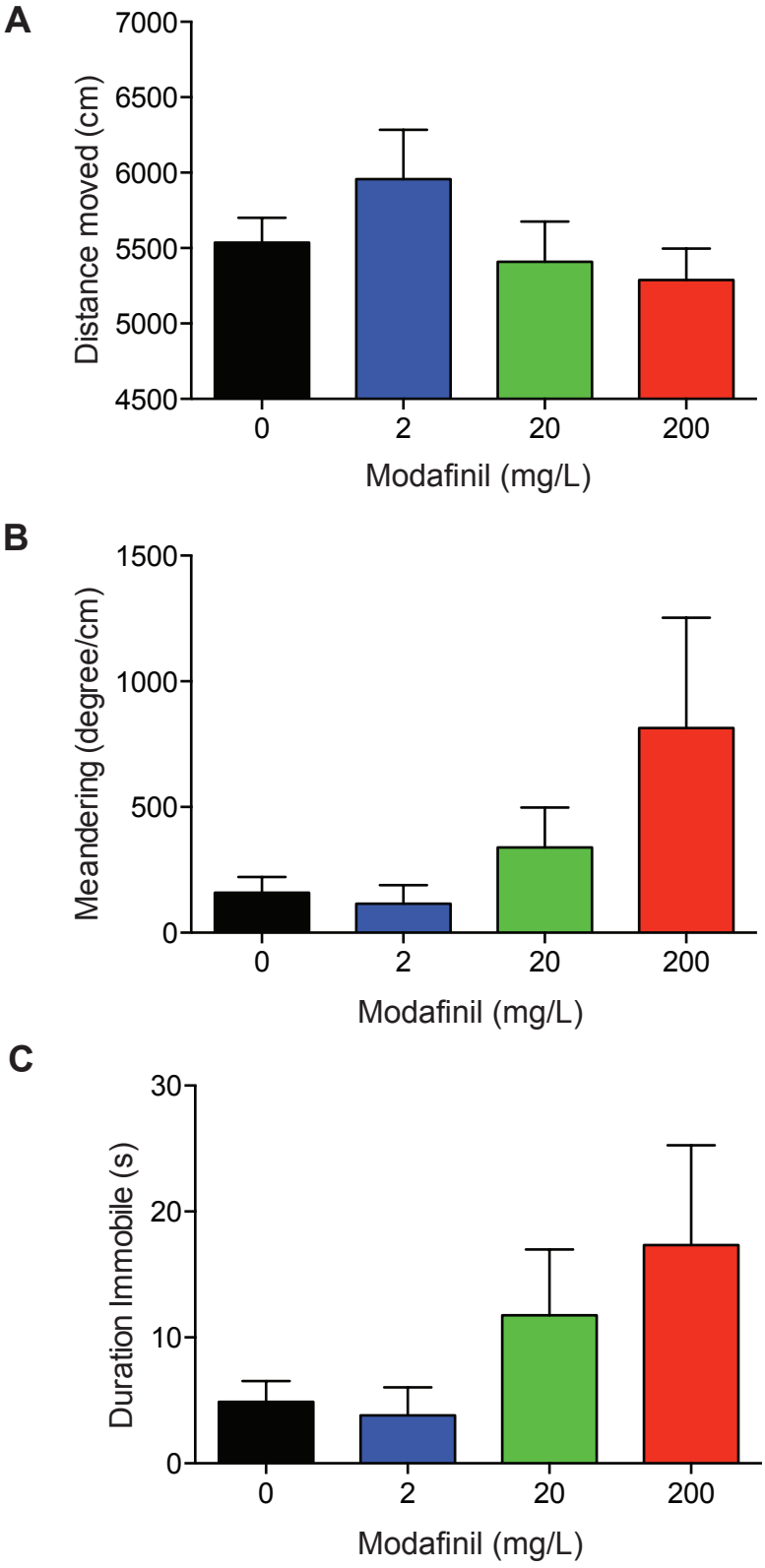

A
Time in thigmotaxis zone (s)
600
500
400
300
*
**
*
0
2
20
200
Modafinil (mg/L)
B
Time in transition zone (s)
200
150
100
50
0
*
*
*
0
2
20
200
Modafinil (mg/L)
C
Time in center zone (s)
15
10
5
0
0
2
20
200
Modafinil (mg/L)

Figure 4


A
7000
6500
6000
5500
5000
4500
Distance moved (cm)
0
2
20
200
Modafinil (mg/L)
B
1500
1000
500
0
Meandering (degree/cm)
0
2
20
200
Modafinil (mg/L)
C
30
20
10
0
Duration Immobile (s)
0
2
20
200
Modafinil (mg/L)