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Effect of Alpha-2 Agonists on the Locomotor Activity in Adult Zebrafish

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Abstract

This study aimed to validate zebrafish as a suitable model for investigating the sedative impact of alpha-2 adrenergic receptor agonists. Zebrafish were exposed to xylazine (500 and 1000 µg/L), clonidine (50 and 500 µg/L), and dexmedetomidine (5 and 50 µg/L), and their locomotor activity was recorded using a Sony 13.6MP digital camera at 5-minute intervals up to 45 min post-exposure. Kinovea software was used to track and analyze the distance travelled and the swimming speed. Xylazine exhibited decreased locomotor activity at 500 µg/L but increased activity at 1000 µg/L, possibly indicating overlapping effects on α-1 receptor adrenergic receptors. Clonidine showed no significant reduction, possibly because of its lower affinity to the zebrafish α-2 receptor compared to the humanα-2 receptor, or could have lower absorption through the skin and gills due to the low logP value of clonidine. Dexmedetomidine significantly decreased the locomotor activity due to its 10 times higher α-2 adrenergic receptor selectivity as compared to xylazine and clonidine. These findings support the use of adult zebrafish as an alternative model to explore the effects of α-2 receptor agonists in drug discovery research.

Keywords: Zebra fish, Alpha-2 agonists, Locomotor activity

The zebrafish, with a clear correlation between neuroanatomical and various physiological features similar to mammals, presents a robust model for drug development, demonstrating sensitivity to pharmacological and environmental manipulations, making it a powerful alternative model for drug research (Rosa *et al.*, 2022). Zebrafish exhibit robust locomotor activity patterns, including swimming and exploration behaviors, which can be used as indicators of drug effects on the central nervous system (Li *et al.*, 2018). The tools required to study this behavior should be inexpensive and accessible to students of academic institutions (Selvaraj *et al.*, 2019a) detection of neurobehavioral disorders and chemical toxicology. The emergence of computational approaches has helped to develop different tools to analyse complex behaviors. Analysis of locomotor behavior helps in understanding the motor neuron disorders like Parkinson's Disease. Although many animal models are available to study the locomotion, adult zebrafish has emerged as a simple and efficient model to study this behavior. An inexpensive and easily customizable tool is required to replace the licensed and expensive set-up to analyse the locomotor behavior.\nNew method\nIn this study we have optimized the ImageJ plugin wrMTrck to analyse motor and non-motor behaviors in adult zebrafish. We have generated a macro to simplify the preprocessing and tracking. Subsequently, we have developed a data analysis sheet to analyse various behavioral end points.\nResults\nWe have successfully developed an inexpensive video acquisition set-up and optimized wrMTrck for adult zebrafish. In order to demonstrate the efficacy of this method, adult zebrafish were injected with MPTP and motor and non-motor behaviors were analysed. Expectedly, MPTP injected fish showed decrease in dopamine

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The study was performed at the Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai.

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level and dat expression level, which subsequently led to locomotor behavioral defects as well as anxiety, a non-motor symptom of PD.\ nComparison with existing method(s. Despite the anatomical differences in the brain between zebrafish and mammals, zebrafish perform similar functions as in mammals because of the similarity of neurotransmitter systems, including adrenergic receptors, and zebrafish have been used to study the effects of mafedine, an alpha-2-agonist, on the novel tank test and anxiety (Sysoev *et al.*, 2019). The locomotor activity of various CNS depressants, including clonidine, in zebrafish has been studied (Gupta *et al.*, 2014); however, comparison of the highly potent alpha-2-agonist dexmedetomidine, which is 10 times more potent than xylazine or clonidine (Gertler *et al.,* 2001)an α2-adrenoceptor agonist, the indications for this class of drugs have continued to expand. In December 1999, dexmedetomidine was approved as the most recent agent in this group and was introduced into clinical practice as a short-term sedative (<24 hours, on locomotor activity in zebrafish is unavailable. Therefore, the current study was undertaken to investigate the differences in the locomotor activity of xylazine, clonidine, and dexmedetomidine to understand the effect of alpha-2-agonistswith varied potencies, which translates into behavioral parameters such as locomotor activity in zebrafish.

Materials and Methods

Animal and Maintenance

Six-month-old adult wild zebrafish were acclimatized for at least two weeks before starting the study. Zebrafish were housed in a four-liter water tank, filled with water at a ratio of 3:1 of tap water to reverse osmosis filtered (RO) water (2.25 liter of tap water, 0.75 liter of RO water) with constant aeration. The utmost care was taken to ensure that all fish were treated humanely. The experiment was conducted during the light phase (3.00 pm-5.00 pm) of the day.

Chemicals and Reagents

Xylazine (Xylaxin 2%, Indian Immunologicals Ltd., India), clonidine (Cloneon 150 µg/mL), and dexmedetomidine (Dextomid 50 µg/mL, Neon Laboratories Ltd., India) were purchased from a veterinary pharmacy. The concentration of each drug was selected based on preliminary experiments conducted in zebrafish (data not shown). The concentrations selected for the study were xylazine (500 and 1000 µg/L), clonidine (50 and 500 µg/L), and dexmedetomidine (5 and 50 µg/L). Equivalent volumes of normal saline were mixed with aquarium water in the control group.

Locomotor Activity Monitoring

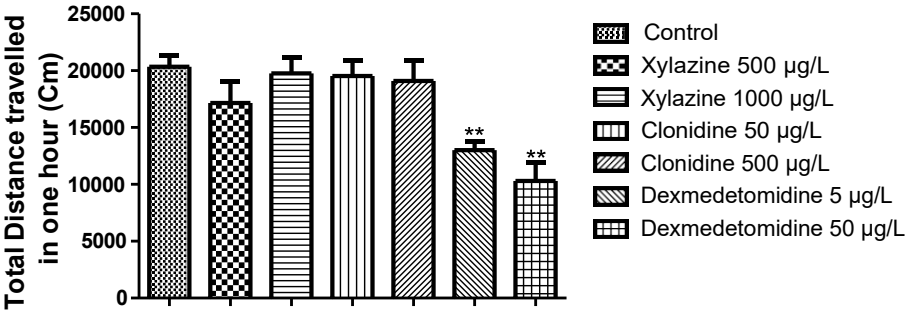
Three zebra fish were subjected to different drug groups at different dose levels dissolved in water. After acclimatization of each fish for 30 min, locomotor activity was recorded for 1 min at 5 min intervals. Pre-exposure recording was conducted for 15 min, and post-exposure recording was conducted for 45 min. For analysis of locomotor activity, distance travelled (cm) was monitored using video tracking software (KINOVEA).

Statistics

The mean and standard error values of distance travelled (cm) and speed (cm/min) were calculated for each drug concentration, and their areas covered over 60 min were compared with the control using one-way analysis of variance (ANOVA) after checking the normality and homogeneity of variance, followed by post-hoc Dunnett's Multiple Comparison Test to compare the treatment group with the control group. Time vs. treatment group interaction was checked with Two-way ANOVA followed by Bonferroni post-hoc tests to identify significant differences between the treatment group and control group at all time points using GraphPad Prism software v5.0.

Results

The effect on locomotor activity in adult zebrafish was evaluated using xylazine (500 and 1000 µg/L), clonidine (50 and 500 µg/L), and dexmedetomidine (5 and 50 µg/L). The locomotor activity of zebrafish was monitored after treatment with drugs and compared with that of the vehicle.



Each bar represents Mean ± SE; **P<0.01

Fig 1: Effect of α-2 agonists on locomotor activity in adult zebrafish

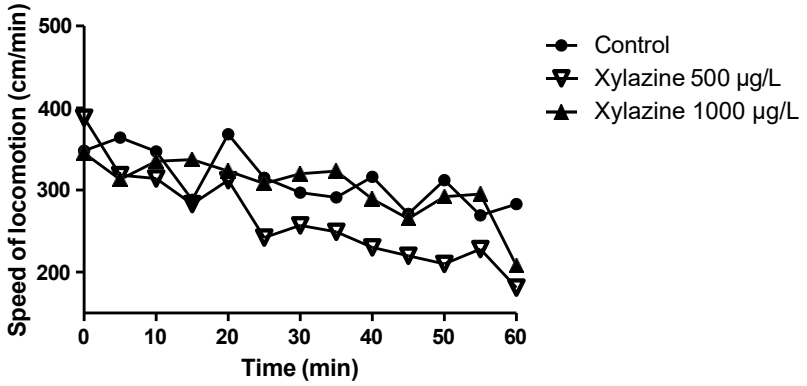


Fig 2: Effect of Xylazine on locomotor activity in adult zebrafish

In the control group, the speed at which the zebrafish travelled was within the range of 271-395 cm/min. When treated with xylazine at a dose of 500 µg/L, there was a reduction in speed (<250cm/min) and the total distance travelled from 25 min onwards. With xylazine at 1000 µg/L, the speed and distance travelled were reduced at the 60thminute compared with the control.

No effect on locomotor activity was observed when zebrafish were treated with clonidine at both 50 µg/L and 500 µg/L concentrations (Fig 4). However, there was a trend for a decrease in locomotor activity from the 35th to 60th min, although this was not statistically significant.

Dexmedetomidine at 5µg/L &50µg/L showed sharp reductions in speed and locomotor activity at 30 and 20 min, respectively. Thereafter, the activity was low throughout the 45 min study period for both concentrations (Fig 4).

The change in the speed of the α-2 agonists at 45 min post-exposure showed a significant reduction with xylazine at 500 µg/L and dexmedetomidine at both concentrations. However, other exposure groups also showed a trend of reduction in speed as compared to the control group (Fig 5 and Table I).

Locomotion tracking plots of zebrafish for α-2 agonists at specified concentrations obtained from the video tracking system (kinovea) are shown in Table II.

Discussion

Adult zebrafish locomotor activity has been used as a model to study the effects of drugs, chemicals, and pranic energy on neurobehavior (Li *et al*., 2018; Pankaj Gupta *et al*., 2014; Selvaraj et al., 2019b) and can be used to compare the potency of various drugs on

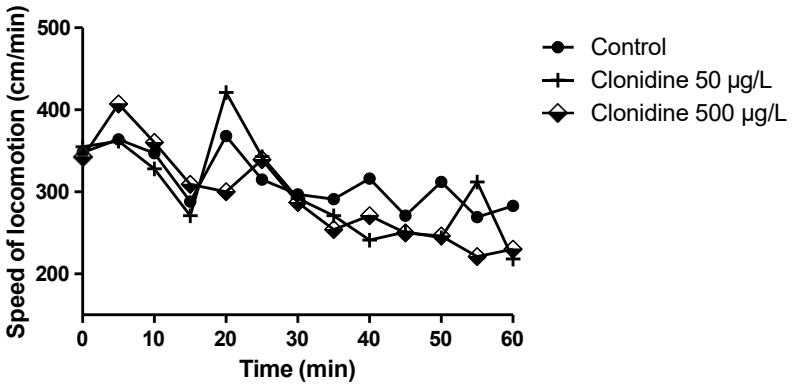


Fig 3: Effect of Clonidine on locomotor activity in adult zebrafish

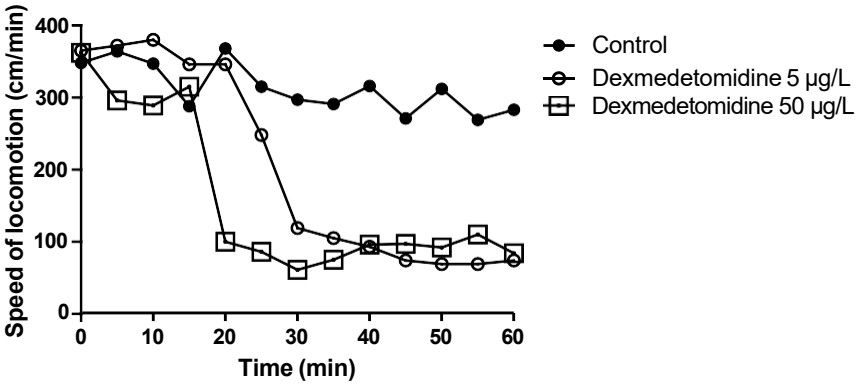
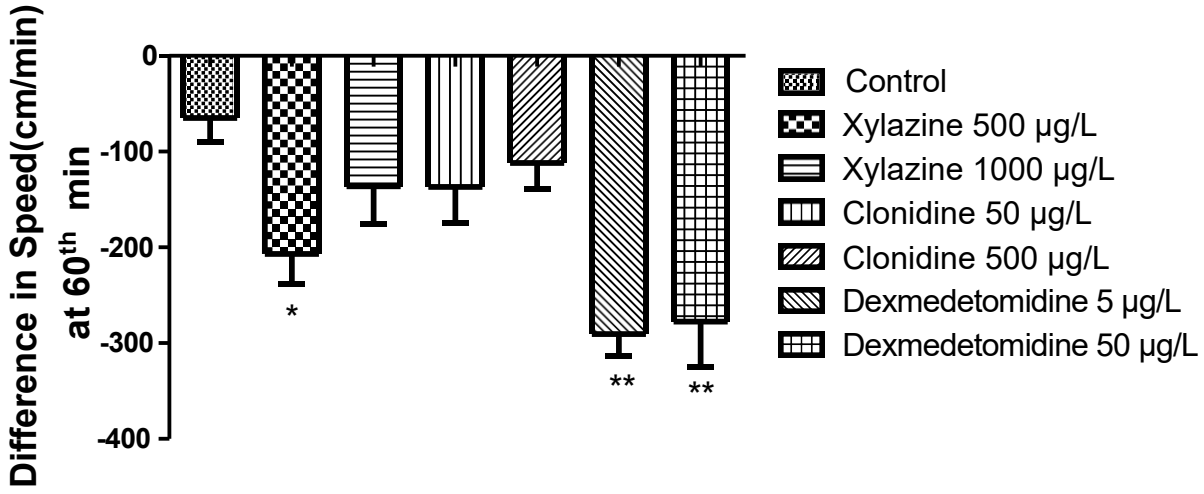


Fig 4: Effect of Dexmedetomidine on locomotor activity in adult zebrafish

locomotor activity within the same class of drugs. The current study was undertaken to identify whether the zebrafish model could be used to assess differences in the potency of α-2 agonists on locomotor activity. Commonly used α-2 agonists, such as xylazine, clonidine, and dexmedetomidine, were selected to study the difference in their selectivity against adrenergic α-2 receptor vs. α-1 receptor, which results in decreased locomotor activity due to muscle relaxant properties (Lemke, 2004). The locomotor activity of zebrafish was tracked using the online free tool Kinovea (https://www.kinovea.org/), which is a video annotation tool. Xylazine at a lower concentration of 500 µg/L showed decreased swimming speed as compared to the control, whereas at 1000 µg/L it showed increased activity, which is an unexpected effect. This could be due to the loss of selectivity of the α-adrenergic receptors (α1 and α2) at higher concentrations, and the increased locomotor activity is due to the overlapping effect on the α1 adrenergic receptor. Clonidine at both concentrations (50 and 500 µg/L) did not show any decrease in locomotor activity, and the exact reason for this is unknown. The absence or reduced activity could be due to the reduced absorption of clonidine in zebrafish, as the logP of clonidine (1.6) was less than that of xylazine and dexmedetomidine (Table III).

It has been reported that the absorption of drugs increases in zebrafish with a higher logP value and the 90th percentile value of logP for drugs absorbed in zebrafish was 5.3 (Long *et al*., 2019). Moreover, clonidine is shown to have nine times less affinity (Ki) with the α2a subtype of zebrafish (89 nM) as compared to the human α2a receptor (10 nM) (Ruuskanen *et al.*, 2005), which explains the low or no activity of clonidine in zebrafish locomotor activity, as the α2a receptor is



Each bar represents Mean ± SE; *P<0.05; **P<0.01

Fig 5: Effect of α-2 agonists on swimming speed at the end of the study, 60th min.

Table I : Effect of α-2 agonists on the speed (cm/min) of zebrafish locomotion

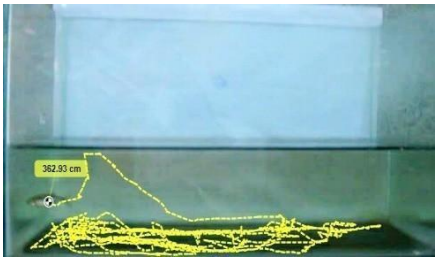
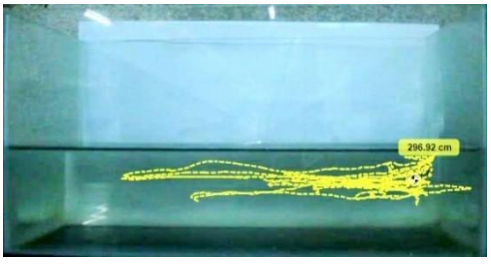
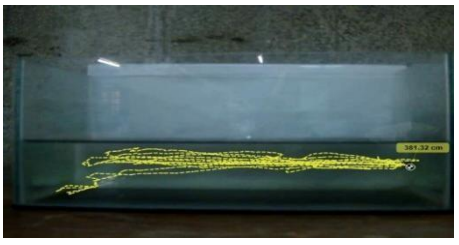
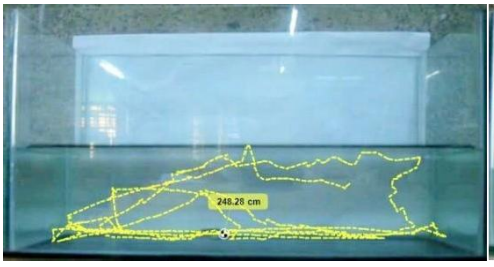
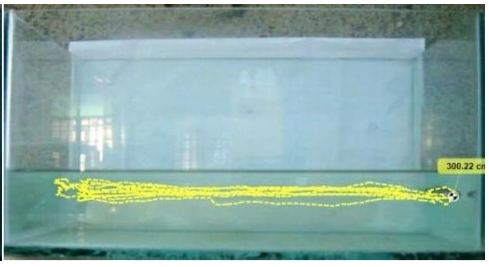
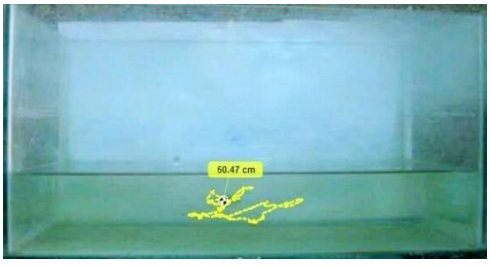
Time (Min)	Control	Xylazine		Clonidine		Dexmedetomidine	
		500 µg/L	1000 µg/L	50 µg/L	500 µg/L	5 µg/L	50 µg/L
0	348 ± 43	388 ± 35	345 ± 66	355 ± 33	342 ± 113	365 ± 56	362 ± 141
5	364 ± 43	318 ± 31	313 ± 89	362 ± 50	407 ± 105	372 ± 57	296 ± 120
10	347 ± 43	314 ± 80	335 ± 76	328 ± 55	360 ± 131	380 ± 95	289 ± 81
15	288 ± 29	283 ± 94	337 ± 126	271 ± 120	309 ± 100	346 ± 29	315 ± 108
20	368 ± 97	312 ± 25	323 ± 66	421 ± 106	300 ± 39	346 ± 40	100 ± 31*
25	315 ± 33	242 ± 66	308 ± 53	343 ± 88	339 ± 48	248 ± 90	86 ± 30*
30	297 ± 24	257 ± 105	320 ± 23	292 ± 73	287 ± 75	119 ± 51*	61 ± 29*
35	291 ± 62	249 ± 92	323 ± 50	271 ± 114	254 ± 65	105 ± 72*	75 ± 40*
40	316 ± 61	230 ± 112	289 ± 55	241 ± 89	271 ± 74	93 ± 65*	96 ± 40*
45	271 ± 62	220 ± 128	265 ± 38	251 ± 107	250 ± 78	74 ± 25*	97 ± 70*
50	312 ± 75	210 ± 122	292 ± 67	244 ± 102	246 ± 55	69 ± 21*	92 ± 53*
55	269 ± 56	228 ± 61	295 ± 72	312 ± 39	221 ± 37	69 ± 17*	110 ± 79*
60	283 ± 79	181 ± 67	208 ± 60	218 ± 113	230 ± 52	74 ± 19*	84 ± 35*

Values are Mean±SEM; Two-way ANOVA *P<0.05 as compared to the control group in Bonferroni post-hoc tests

involved in motor behavior in mice (Hunter *et al*., 1997; Sysoev *et al.*, 2019). Dexmedetomidine, an active dextroisomer of medetomidine, shows selective α-2 adrenoceptor agonism and is 10 times more selective than xylazine and clonidine (Virtanen *et al*., 1988). This higher selectivity was reflected in the higher potency of dexmedetomidine in the locomotor activity of zebrafish. However, all three α-2 agonists showed a reduction in locomotor activity after 60 min of exposure, which could be due to the delay in the attainment of a steady state of drug concentration in zebrafish as the drug is mixed in the water and the absorption of the drug occurs through the skin and gills of the zebrafish (Morikane *et al*., 2020).Conclusion

The present study was initiated with the aim of utilizing zebrafish as a model organism to evaluate the potency of adrenergic α-2 receptor agonists including xylazine,

Table II : Track of zebrafish swim in the tank of control and α-2 agonists at specified concentrations

Control		
Xylazine		
	500 µg/L	1000 µg/L
Clonidine		
	50 µg/L	500 µg/L
Dexmedetomidine		
	5 µg/L	50 µg/L

clonidine, and dexmedetomidine. The results of the study revealed that xylazine and clonidine were less selective towards the α-2 receptor than dexmedetomidine, which exhibits high selectivity towards the α-2 receptor in zebrafish. The study also demonstrated a significant reduction in motor activity with dexmedetomidine and xylazine at low concentrations, whereas no reduction was observed with clonidine. Therefore, zebrafish can be utilized as a model system to study differences in α-2 receptor activity. However, additional research is necessary to establish a dose-response relationship between in-water and intraperitoneal exposure to various α-2 receptor agonists or antagonists.

References

Gertler, R., Brown, H. C., Mitchell, D. H., and Silvius, E. N. (2001). Dexmedetomidine: A novel sedative-anal-

Table III: Selectivity of α-2 Vs α-1 and Lipophilicity of α-2 agonists

Drugs	α-2 Vs α-1	LogP
Xylazine	160:1	4.5
Clonidine	220:1	1.6
Dexmedetomidine	1620:1	2.8

gesic agent. *Proceedings (Baylor University. Medical Center)*, **14**(1) : 13–21.

Hunter, J. C., Fontana, D. J., Hedley, L. R., Jasper, J. R., Lewis, R., Link, R. E., Secchi, R., Sutton, J., and Eglen, R. M. (1997). Assessment of the role of α2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice. *British Journal of Pharmacology*, **122**(7) : 1339–1344. https://doi.org/10.1038/sj.bjp.0701520

Lemke, K. A. (2004). Perioperative use of selective alpha-2 agonists and antagonists in small animals. *The Canadian Veterinary Journal*, **45**(6) : 475–480.

Li, F., Lin, J., Liu, X., Li, W., Ding, Y., Zhang, Y., Zhou, S., Guo, N., and Li, Q. (2018). Characterization of the locomotor activities of zebrafish larvae under the influence of various neuroactive drugs. *Annals of Translational Medicine*, **6**(10) : 173–173. https://doi.org/10.21037/atm.2018.04.25

Long, K., Kostman, S. J., Fernandez, C., Burnett, J. C., and Huryn, D. M. (2019). Do Zebrafish Obey Lipinski Rules? *ACS Medicinal Chemistry Letters*, **10**(6) : 1002–1006. https://doi.org/10.1021/acsmedchemlett.9b00063

Morikane, D., Zang, L., and Nishimura, N. (2020). Evaluation of the Percutaneous Absorption of Drug Molecules in Zebrafish. *Molecules*, **25**(17) : 3974. https://doi.org/10.3390/molecules25173974

Pankaj Gupta, Shailaja B Khobragade, Vyas M Shingatgeri, and Srinivisan M Rajaram. (2014). Assessment of locomotion behavior in adult Zebrafish after acute exposure to different pharmacological reference compounds. *Drug Development and Therapeutics*, **5**(2) : 127–133.

Rosa, J. G. S., Lima, C., and Lopes-Ferreira, M. (2022). Zebrafish Larvae Behavior Models as a Tool for Drug Screenings and Pre-Clinical Trials: A Review. *International Journal of Molecular Sciences*, **23**(12) : 6647. https://doi.org/10.3390/ijms23126647

Ruuskanen, J. O., Laurila, J., Xhaard, H., Rantanen, V.-V., Vuoriluoto, K., Wurster, S., Marjamäki, A., Vainio, M., Johnson, M. S., and Scheinin, M. (2005). Conserved structural, pharmacological and functional properties among the three human and five zebrafish α2-adrenoceptors. *British Journal of Pharmacology*, **144**(2) : 165–177. https://doi.org/10.1038/sj.bjp.0706057

Selvaraj, V., Venkatasubramanian, H., Ilango, K., and Santhakumar, K. (2019a). A simple method to study motor and non-motor behaviors in adult zebrafish. *Journal of Neuroscience Methods*, **320** : 16–25. https://doi.org/10.1016/j.jneumeth.2019.03.008

Selvaraj, V., Venkatasubramanian, H., Ilango, K., and Santhakumar, K. (2019b). A simple method to study motor and non-motor behaviors in adult zebrafish. *Journal of Neuroscience Methods*, **320** : 16–25. https://doi.org/10.1016/j.jneumeth.2019.03.008

Sysoev, Y. I., Meshalkina, D. A., Petrov, D. V., Okovityi, S. V., Musienko, P. E., and Kalueff, A. V. (2019). Pharmacological screening of a new alpha-2 adrenergic receptor agonist, mafedine, in zebrafish. *Neuroscience Letters*, **701** : 234–239. https://doi.org/10.1016/j.neulet.2019.03.001

Virtanen, R., Savola, J.-M., Saano, V., and Nyman, L. (1988). *Characterization of the selectivity, specificity and potency of medetomidine as an a2-adrenoceptor agonist*. **150** : 9–14.