BIOLOGY LETTERS

rsbl.royalsocietypublishing.org

Research

Cite this article: Hamilton TJ, Tresguerres M, Kline DI. 2017 Dopamine D1 receptor activation leads to object recognition memory in a coral reef fish. *Biol. Lett.* **13**: 20170183.
http://dx.doi.org/10.1098/rsbl.2017.0183

Received: 22 March 2017
Accepted: 14 June 2017

Subject Areas:
behaviour, cognition, neuroscience

Keywords:
dopamine, damselfish, spatial memory, object recognition

Author for correspondence:
Trevor J. Hamilton
e-mail: trevorjameshamilton@gmail.com

†These authors contributed equally to this study.

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3820747.

THE ROYAL SOCIETY PUBLISHING

Marine biology

Dopamine D1 receptor activation leads to object recognition memory in a coral reef fish

Trevor J. Hamilton[1,2], Martin Tresguerres[3,†] and David I. Kline[3,4,†]

[1]Department of Psychology, MacEwan University, Edmonton, Alberta, Canada T5 J 4S2
[2]Neuroscience and Mental Health Institute, University of Alberta, Edmonton, Alberta, Canada T6G 2H7
[3]Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
[4]Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Panamá, República de Panamá

TJH, 0000-0002-8990-8247; MT, 0000-0002-7090-9266

Object recognition memory is the ability to identify previously seen objects and is an adaptive mechanism that increases survival for many species throughout the animal kingdom. Previously believed to be possessed by only the highest order mammals, it is now becoming clear that fish are also capable of this type of memory formation. Similar to the mammalian hippocampus, the dorsolateral pallium regulates distinct memory processes and is modulated by neurotransmitters such as dopamine. Caribbean bicolour damselfish (*Stegastes partitus*) live in complex environments dominated by coral reef structures and thus likely possess many types of complex memory abilities including object recognition. This study used a novel object recognition test in which fish were first presented two identical objects, then after a retention interval of 10 min with no objects, the fish were presented with a novel object and one of the objects they had previously encountered in the first trial. We demonstrate that the dopamine D_1-receptor agonist (SKF 38393) induces the formation of object recognition memories in these fish. Thus, our results suggest that dopamine-receptor mediated enhancement of spatial memory formation in fish represents an evolutionarily conserved mechanism in vertebrates.

1. Introduction

Coral reefs constitute a very complex environment with abundant holes, crevasses and eipifauna where fish hide, forage and mate. To be able to navigate within the reef and return to a specific territory, feeding ground or shelter, fish need to be able to recognize familiar cues, some of which are visual.

In vertebrate animals including rodents, humans [1] and fish [2], memory formation can be enhanced by the neurotransmitter dopamine. Through this mechanism, a normally unexciting stimulus is made salient to the organism with the concurrent release of dopamine in the neuronal reward/novelty system [3].

In areas of the brain that are responsible for the formation of spatial memories such as the mammalian hippocampus, dopamine enhances long-term changes in synaptic strength via induction of long-term potentiation [1]. Dopamine binds to five classes of dopamine G-protein coupled receptors (GPCRs), which modulate Gα-proteins and downstream cAMP production by transmembrane adenylyl cyclases. In turn, cAMP mediates protein kinase A-dependent phosphorylation of target proteins that determine cellular responses. There are two classes of dopamine-responsive GPCRs; D_1-class (D_1 and D_5) and D_2-class receptors (D_2, D_3 and D_4) [4,5]. D_1-class receptors activate $G\alpha_{s/olf}$

GPCRs and increase cAMP production by adenylyl cyclases, while D_2-class receptors activate $G\alpha_{i/o}$ GPCRs, inhibiting cAMP production. D_1-class receptors are expressed more widely in the brain compared to the D_2-class and both are present postsynaptically [4,5]. D_1 and D_2 receptors have been reported in zebrafish [6,7], and the dopaminergic system is known to partially regulate reward, motivation, sociality and response to novelty in teleost fish [8–14]. Additionally, the administration of D_1 receptor agonists has recently been shown to enhance learning based tasks in cleaner wrasse (*Labroides Dimidiatus*) [2] and zebrafish [15].

In this study, we used the Caribbean bicolour damselfish (*Stegastes partitus*; Poey, 1868 [16]), a small (approx. 10 cm), planktivorous pomacentrid that inhabits shallow patch reefs and is extremely territorial [17,18]. Like other damselfish, *S. partitus* settle on small offshore reefs where they spawn and lay eggs then remain near their nest for long periods of time [19], thus making them a candidate to possess object recognition memory. Novel object recognition/preference tests have been a hallmark of memory studies in rodent literature for the last three decades [20,21]. This one-trial learning and memory test involves the presentation of two identical objects, followed by a retention interval in which the animal is situated in an arena with no stimuli, prior to the presentation of one previously seen object and one novel object [20]. An increase in the proportion of time around the novel object is a proxy for object recognition memory in rodent studies because they have recognized the familiar object and prefer to explore the novel one [15]. Zebrafish, on the other hand, are fearful of the novel stimuli and preference for the familiar object is used to quantify object recognition [17]. To date, there have been no studies on the memory capacities of the Caribbean damselfish. Here, we used the novel object recognition test with a retention interval of 10 min [22]. The dopamine D_1-receptor agonist (SKF 38393), previously shown to enhance learning in cleaner wrasse [1] and zebrafish [15], was administered prior to the presentation of the identical objects to determine whether this would enhance object recognition/preference. Since dopamine D_1-agonists have been previously shown to increase locomotion in zebrafish [23], we also monitored distance travelled. Our results support the involvement of dopamine D_1-receptors in object recognition memory formation in *S. partitus*.

2. Material and methods

(a) Subjects and pharmacology

Stegastes partitus were caught near the Smithsonian Tropical Research Institute's (STRI) Caribbean field station in Bocas del Toro, Panama. Fish were individually housed in plastic 3.5 l containers provided with flow through seawater (29.5 ± 0.5°C) and one piece of white polyvinyl chloride (PVC) piping to act as a hiding place for at least 3 days prior to testing. Fish were fed once daily with sinking tropical food pellets (Omega One sinking mini-pellets, OmegaSea Ltd, Painesville, OH, USA) and were not fed prior to testing on the day of experimentation. The STRI aquarium has a transparent roof and no walls, which maintained the natural approximately 12 D : 12 N cycle. The selective D_1 receptor agonist (SKF 38393; Abcam, Eugene, OR, USA) was dissolved in fresh water and DMSO (Sigma) to a final concentration of 10 mg l^{-1} based on previous studies [15,24]. Control and control + DMSO solutions contained the same amount of freshwater as in the drug solutions. There were no differences in weight (control: 3.90 ± 0.36 g, $n = 8$; SKF: 3.04 ± 0.63 g, $n = 8$, $p = 0.2552$, unpaired t-test) or length (control: 5.4 ± 0.2 cm, $n = 8$; SKF: 5.1 ± 0.3 cm, $n = 8$, $p = 3876$) between the control and D_1 agonist groups.

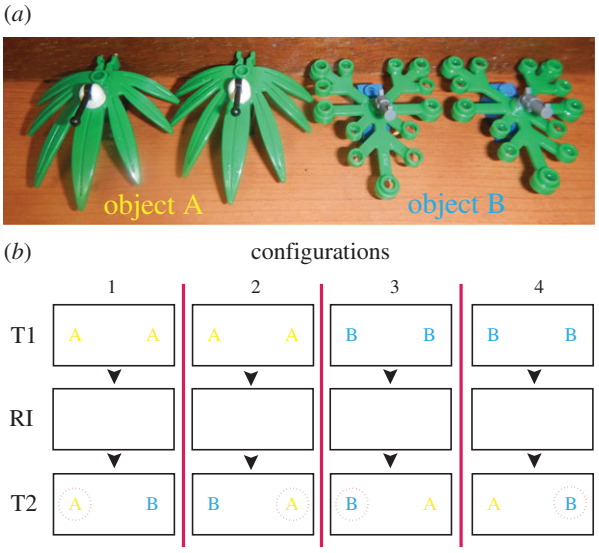

Figure 1. Experimental set-up. (*a*) Lego 'object A' and 'object B'. (*b*) Each fish underwent a testing protocol in which they were exposed to two identical objects in trial 1 (T1), then were exposed to no objects for the 10 min retention interval (RI), and finally were exposed to one familiar (circled) and one novel object in trial 2 (T2). (Online version in colour.)

(b) Experimental testing

Testing took place between 09.00 and 17.00 h. See the electronic supplementary material (detailed in Material and methods) for full testing procedure and statistics. Briefly, damselfish from all groups were first individually placed into the dosing tank for 10 min, then into the experimental tank containing two identical objects for 10 min (trial 1; figure 1). Next, they were placed into a holding tank which was the same size as the experimental tank for 10 min (retention interval, 'RI'). Finally, fish were placed back into the experimental tank with one previously seen object replaced with a novel object (trial 2). Familiar object preference index 1 (FOP1) and familiar object preference index 2 (FOP2) preference indices were calculated by comparing the time fish spent in zones containing each object, based on previous studies [15–17], with positive values indicating a preference for the familiar object (see the electronic supplementary material for full details). Control fish ($n = 4$) and control + DMSO fish ($n = 4$) showed no significant difference in preference for either object (Disc1; $t(7) = 0.9714$, $p = 0.3689$) so these groups were combined.

3. Results

Damselfish administered the D_1-receptor agonist (10 mg l^{-1}) demonstrated a significant preference for the familiar object (SKF: $t(7) = 2.627$, $p = 0.0341$, $n = 8$, one sample t-test, difference from zero), whereas the control fish displayed no clear preference for the familiar or novel object (control: $t(7) = 0.7544$, $p = 0.4752$, $n = 8$, one sample t-test, difference from zero) and there was a significant difference in preference between the two groups ($t(14) = 2.358$, $p = 0.0335$; unpaired t-test; figure 2). The same results were obtained with the FOP2 index; the SKF group displayed a clear familiar object

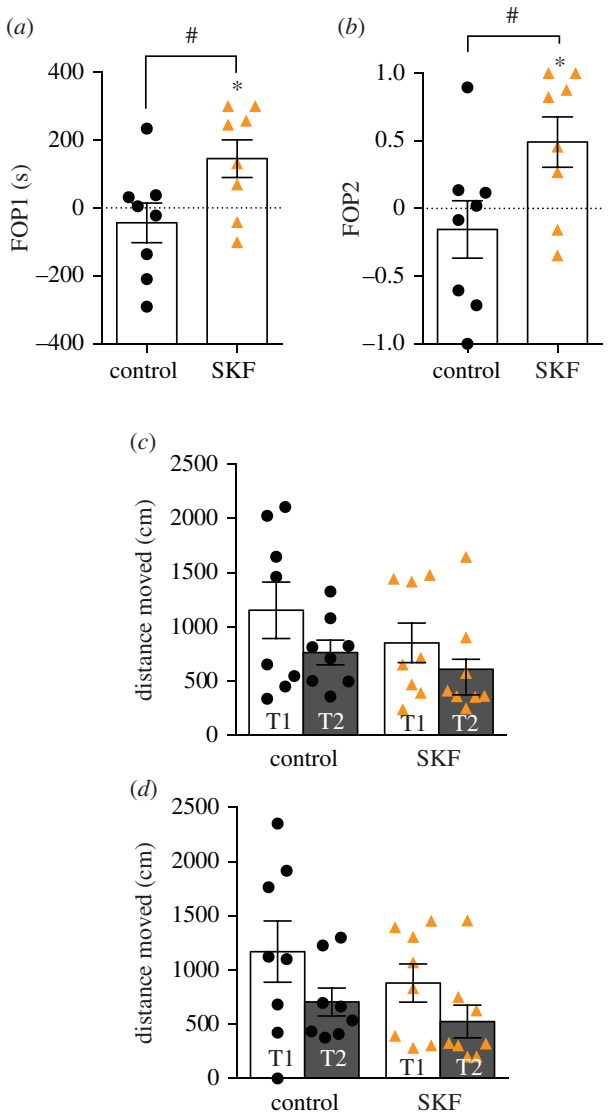

Figure 2. Object preference and locomotion. (*a*) Familiar object preference index (FOP1), and (*b*) familiar object preference index 2 (FOP2). The asterisk (*) indicates a significant difference from zero (one sample *t*-test, difference from zero), indicative of a significant preference for the familiar object. The hash symbol (#) indicates a significant difference in preference between the control and SKF groups unpaired *t*-test. (*c*) Total distance moved in the first 5 min of trial 1 (T1) and trial 2 (T2); (*d*) total distance moved in the last 5 min T1 and T2. No statistical differences were detected in any case (Kruskal–Wallis test) ruling out an effect of SKF on locomotion. Filled circles and squares represent individual fish in the control and SKF groups, respectively. (Online version in colour.)

preference ($t(7) = 2.631$, $p = 0.0338$, one sample *t*-test, difference from zero), the controls showed no preference ($t(7) = 0.7291$, $p = 0.4896$, one sample *t*-test, difference from zero), and there was a significant difference between these groups ($t(14) = 2.284$, $p = 0.0385$, unpaired *t*-test; figure 2). There was no significant difference in distance moved between either group in trial 1 or trial 2 in the first 5 min of each trial ($H(4) = 3.452$, $p = 0.3271$, Kruskal–Wallis test; figure 2) or in the last 5 min of each trial ($H(4) = 4.588$, $p = 0.2046$, Kruskal–Wallis test; figure 2), or in the entire 10 min trial ($H(4) = 4.776$, $p = 0.1890$, Kruskal–Wallis test, data not shown) suggesting that the administration of SKF was sufficient to increase memory formation but had no effect on locomotion.

4. Discussion

A flexible object recognition memory system is a valuable learning mechanism that allows organisms to recall ecologically relevant stimuli important for survival. The current study tested the effect of dopamine on memory formation in damselfish *S. partitus*. Control damselfish spent the same amount of time in the proximity of the two objects, indicating they did not recognize or care for either object. However, pharmacological activation of dopamine D_1 receptors by SKF 38393 prior to exposure to the first two identical objects resulted in a clear preference for the familiar object during the second exposure. Thus, exposure to a normally mundane object after D_1 receptor activation rendered the object salient. This is the first demonstration of object recognition memory in a marine fish using a laboratory test and is consistent with many studies demonstrating diverse and complex processing of spatial locations [25]. In their native environment, the dopamine reward system might allow damselfish to recognize a beneficial reef or location such as their territory, profitable food source, or location of receptive mates for reproduction and encode this information for future use.

These results support recent studies demonstrating a modulatory influence of dopamine on associative learning [15] and latent learning [24] in zebrafish, and learning decision-making tasks in cleaner wrasses [2]. Our results demonstrate that under these experimental conditions damselfish can develop object recognition memory for at least a 10-min span after D_1 agonist treatment. Interestingly, damselfish did not develop memory formation in the control conditions. Importantly, these fish were held at the research station for 3 days before testing. This could have resulted in increased stress relative to their natural reef environment, which could have altered their ability to form memories. Our testing protocol introduced fish to objects for 10 min and it is likely that in their natural habitat, with repeated learning occurring, their object recognition memory span is much greater than minutes and lasts for months if not years. Future experiments could use shorter and longer retention intervals, and use dopamine D_2 receptor agonists and antagonists to further characterize the role of dopamine in memory formation in this species.

Ethics. The experimental protocol (2016-1020-2019-A1) was approved by STRI Animal Care and Use Committee in compliance with the IACUC standards for animal care and use in research.

Data accessibility. Data are included in the electronic supplementary material.

Authors' contributions. T.J.H. conceived the study, collected the data, performed the analyses, wrote and edited the manuscript. T.J.H., D.I.K. and M.T. contributed to data interpretation, writing and editing, and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests. The authors declare no competing interests.

Funding. Funded by NSERC no. 04843 and a project grant from MacEwan University to T.J.H., and NSF OCE no. 1538495 to D.I.K. and M.T.

Acknowledgements. The authors thank the personnel at the Smithsonian Tropical Research Institute, and Dr Michele Pierotti who provided advice about local Caribbean fish.

References

1. Hamilton TJ, Wheatley BM, Sinclair DB, Bachmann M, Larkum ME, Colmers WF. 2010 Dopamine modulates synaptic plasticity in dendrites of rat and human dentate granule cells. *Proc. Natl Acad. Sci. USA* **107**, 18 185–18 190. (doi:10.1073/pnas.1011558107)
2. Messias JPM, Santos TP, Pinto M, Soares MC. 2016 Stimulation of dopamine D1 receptor improves learning capacity in cooperating cleaner fish. *Proc. R. Soc. B* **283**, 20152272. (doi:10.1098/rspb.2015.2272)
3. Lisman JE, Grace AA. 2005 The hippocampal–VTA loop: controlling the entry of information into long-term memory. *Neuron* **46**, 703–713. (doi:10.1016/j.neuron.2005.05.002)
4. Beaulieu J-M, Espinoza S, Gainetdinov RR. 2015 Dopamine receptors IUPHAR Review 13. *Br. J. Pharmacol.* **172**, 1–23. (doi:10.1111/bph.12906)
5. Beaulieu J-M, Gainetdinov RR. 2011 The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* **63**, 182–217. (doi:10.1124/pr.110.002642)
6. Li P, Shah S, Huang L, Carr AL, Gao Y, Thisse C, Thisse B, Li L. 2007 Cloning and spatial and temporal expression of the zebrafish dopamine D1 receptor. *Dev. Dyn.* **236**, 1339–1346. (doi:10.1002/dvdy.21130)
7. Boehmler W, Obrecht-Pflumio S, Canfield V, Thisse C, Thisse B, Levenson R. 2004 Evolution and expression of D2 and D3 dopamine receptor genes in zebrafish. *Dev. Dyn.* **230**, 481–493. (doi:10.1002/dvdy.20075)
8. Northcutt RG. 2006 Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *J. Comp. Neurol.* **494**, 903–943. (doi:10.1002/cne.20853)
9. Northcutt RG. 1995 The forebrain of gnathostomes: in search of a morphotype. *Brain Behav. Evol.* **46**, 275–318. (doi:10.1159/000113279)
10. Rink E, Wullimann MF. 2002 Connections of the ventral telencephalon and tyrosine hydroxylase distribution in the zebrafish brain (*Danio rerio*) lead to identification of an ascending dopaminergic system in a teleost. *Brain Res. Bull.* **57**, 385–387. (doi:10.1016/S0361-9230(01)00696-7)
11. Rink E, Wullimann MF. 2001 The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain Res.* **889**, 316–330. (doi:10.1016/S0006-8993(00)03174-7)
12. De Serrano AR, Fong C, Rodd FH. 2016 Effects of methylphenidate on responses to novelty in a teleost fish (*Poecilia reticulata*). *Behav. Brain Res.* **302**, 53–59. (doi:10.1016/j.bbr.2016.01.021)
13. Saif M, Chatterjee D, Buske C, Gerlai R. 2013 Sight of conspecific images induces changes in neurochemistry in zebrafish. *Behav. Brain Res.* **243**, 294–299. (doi:10.1016/j.bbr.2013.01.020)
14. Vindas MA *et al.* 2014 Coping with unpredictability: dopaminergic and neurotrophic responses to omission of expected reward in Atlantic salmon (*Salmo salar* L.). *PLoS ONE* **9**, e85543. (doi:10.1371/journal.pone.0085543)
15. Naderi M, Jamwal A, Chivers DP, Niyogi S. 2016 Modulatory effects of dopamine receptors on associative learning performance in zebrafish (*Danio rerio*). *Behav. Brain Res.* **303**, 109–119. (doi:10.1016/j.bbr.2016.01.034)
16. Poey R. 1869 *The record of zoological literature*. London, UK: J. van Voorst.
17. Schmale MC. 1981 Sexual selection and reproductive success in males of the bicolor damselfish, *Eupomacentrus partitus* (Pisces: Pomacentridae). *Anim. Behav.* **29**, 1172–1184. (doi:10.1016/S0003-3472(81)80069-3)
18. Allen GR. 1991 *Damselfishes of the world*. Melle, Germany: Mergus.
19. Rankin TL, Sponaugle S. 2014 Characteristics of settling coral reef fish are related to recruitment timing and success. *PLoS ONE* **9**, e108871. (doi:10.1371/journal.pone.0108871)
20. Ennaceur A, Delacour J. 1988 A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav. Brain Res.* **31**, 47–59. (doi:10.1016/0166-4328(88)90157-X)
21. Antunes M, Biala G. 2012 The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn. Process* **13**, 93–110. (doi:10.1007/s10339-011-0430-z)
22. May Z, Morrill A, Holcombe A, Johnston T, Gallup J, Fouad K, Schalomon M, Hamilton TJ. 2016 Object recognition memory in zebrafish. *Behav. Brain Res.* **296**, 199–210. (doi:10.1016/j.bbr.2015.09.016)
23. Irons TD, Kelly PE, Hunter DL, Macphail RC, Padilla S. 2013 Acute administration of dopaminergic drugs has differential effects on locomotion in larval zebrafish. *Pharmacol. Biochem. Behav.* **103**, 792–813. (doi:10.1016/j.pbb.2012.12.010)
24. Naderi M, Jamwal A, Ferrari MCO, Niyogi S, Chivers DP. 2016 Dopamine receptors participate in acquisition and consolidation of latent learning of spatial information in zebrafish (*Danio rerio*). *Prog. Neuropsychopharmacol. Biol. Psychiatry* **67**, 21–30. (doi:10.1016/j.pnpbp.2016.01.002)
25. Odling-Smee L, Simpson SD, Braithwaite VA. 2011 The role of learning in fish orientation. In *Fish cognition and behavior* (eds C Brown, K Laland, J Krause), pp. 166–185. Oxford, UK: Wiley-Blackwell.