

Arginine vasotocin modulates associative learning in a mutualistic cleaner fish

Sónia C. Cardoso^{1,2} · Redouan Bshary³ · Renata Mazzei^{2,3} · José R. Paitio¹ · Rui F. Oliveira^{1,4,5} · Marta C. Soares²

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Abstract Social environmental complexity induces structural and biochemical changes in animals' brains, which are linked to the improvement of animals' learning abilities. The nonapeptides from the arginine vasopressin (AVP) family (arginine vasotocin, AVT, in non-mammals) play a significant role in the regulation of social behavior, particularly in the formation of social memories and individual recognition. Moreover, the role of AVT in the regulation of interspecific interactions has only recently started to be addressed in the context of cleaner fish mutualisms and learning. Variance in the distribution of AVP receptor expression, which is linked to distinct neural systems (related to the dorsolateral and the dorsomedial telencephalon), is known to be implicated in differences in individual learning processes. Here we asked if the associative learning performance of the Indo-Pacific bluestreak cleaner wrasse (*Labroides dimidiatus*) is regulated by AVT. We tested the influence of AVT upon the cleaners' ability to solve two different problems (cue and place

discrimination tasks) that in principle differ in ecological relevance and are associated with two different memory systems. We found that AVT affected the learning competence of cleaners differently between tasks, as individual performance showed distinct response selectivity to AVT dosage levels. However, only in the ecologically relevant task was their learning response improved by blocking AVT via treatment with the antagonist Manning compound. Our findings demonstrate that AVT pathways, which are implicated in the regulation of interspecific behavior (i.e., a cleaner's willingness to seek interactions with clients), are also linked to individual learning ability in the context of mutualistic behavior, and in tune with socio-ecological demands.

Keywords Cleaner fish · Learning abilities · *Labroides dimidiatus* · Neuropeptides · Arginine vasotocin · Socio-ecological demands

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✉ Marta C. Soares
marta.soares@cibio.up.pt

¹ Unidade de Investigação em Eco-Etologia, ISPA – Instituto Universitário, Lisboa, Portugal

² CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

³ Eco-Ethologie, Institut de Biologie, Université de Neuchâtel, Neuchâtel, Switzerland

⁴ Integrative Behavioural Biology Group, Instituto Gulbenkian de Ciência, Oeiras, Portugal

⁵ Champalimaud Neuroscience Programme, Champalimaud Foundation, Lisboa, Portugal

Introduction

In cooperative contexts, learning and memory are prerequisites that enable individuals to decide where, when, and how to invest without being cheated by their counterparts (Brosnan et al. 2010). These decision rules are also imperative to the process of choosing among potential partners and to determine the course of action of each interaction. All of these behavioral modules require distinct discriminative abilities that rely on specific types of learning and memory and may be underlined by different anatomical and neurochemical circuits in the brain. Recent empirical studies show that the neural basis of learning and memory processes is identical between teleost fish and other vertebrates, namely mammals (Salas et al. 2006). Like tetrapods, teleost fish not only rely on egocentrically referenced mechanisms for orienting, based

on stimulus-response simple associations, but also are able to use allocentric map-like representation of the environment (Nadel 1991; Broglio et al. 2003; Salas et al. 2003). In mammals, the pallial amygdala is linked to emotional learning and memory, whereas the hippocampus is involved in spatial, contextual, or relational memory (Portavella et al. 2002). In teleost fish, evidence shows that some cognitive abilities might be related to the forebrain, specifically to the dorsolateral (DI) and the dorsomedial (Dm) telencephalon, which are most likely homologs of the hippocampus and amygdala of mammals, respectively (Northcutt 1995; Salas et al. 1996a, b; López et al. 2000a, b; Wullimann and Mueller 2004). For example, training goldfish in a spatial context produces a significant and selective spatial learning-related increase of protein synthesis of DI neurons (Vargas et al. 2000). Likewise, lesion studies show that DI lesions cause impairments in place learning and memory in goldfish when trained in a plus maze surrounded by distal visual cues (Rodríguez et al. 2002). The involvement of DI in spatial cognition seems to be extremely selective, as damage to this area does not affect cue learning or other egocentric strategies (Salas et al. 1996a; López et al. 2000a).

Apart from knowing the neural mechanisms involved in learning, it is also important to study how these mechanisms are modulated by hormones and neuromodulators that will occur at different frameworks and may be species- or context-specific, depending for instance on the amount of receptors in place to translate each signal. Given their previously demonstrated role in the regulation of social behavior (Goodson and Bass 2001; Lim and Young 2006; Goodson and Thompson 2010), nonapeptides of the arginine vasopressin (AVP) family (arginine vasotocin, AVT, in non-mammals) are major candidate modulators of socially relevant learning, which for instance occurs in mutualistic relationships. For example, the central behavioral actions of AVT/AVP are mainly mediated by its V1a receptor subtype in both mammals and non-mammals (Everts and Koolhaas 1999; Lema 2010; Lema et al. 2010). These central actions are vital for the formation of social memories and for individual recognition in mammals (Lim and Young 2006). In fish, AVT has been implicated in both the regulation of reproductive and aggressive behaviors (e.g., promotion of territorial behavior (Santangelo and Bass 2006, 2010), changes in courtship behaviour (Carneiro et al. 2003; Lema and Nevitt 2004), social status (Semsar et al. 2001), and pair formation (Oldfield and Hofmann 2011)) and in the regulation of interspecific cooperative behavior in cleaning mutualisms and in the learning to feed against preference (Soares et al. 2012; Mendonça et al. 2013; Cardoso et al. 2015a, b). It was found that AVT administration caused a decrease of interspecific cleaning interactions, while its antagonist (Manning compound) had opposite effects in mediating a rise in cleaners' dishonesty (Soares et al. 2012). Indeed, by blocking the V1a receptors, cleaners seemed to clean more and care less about maintaining good service

quality (Soares et al. 2012). But, most importantly, in a learning task, Cardoso and colleagues (2015a) found that the experimental transient higher dosage of AVT led to a decrease of cleaners' willingness to feed against their preference, while IT and AVT antagonists had no significant effects. Nevertheless, the precise role of AVT on cognitive learning abilities underlying cooperative behavior in fish warrants further investigation.

In this paper, we asked if the same neuropeptide that was implicated in the regulation of interspecific behavior was also linked to individual learning ability in the context of their mutualistic behavior. For this purpose, we used the cleaner wrasse *Labroides dimidiatus* to study AVT's effects on the learning of two tasks, distinct in terms of socio-ecological relevance. These cleaner wrasses live on coral reefs throughout the tropical Indo-Pacific and feed exclusively on other visiting reef fish (usually referred as client fish) ectoparasites, mucus, and scales (Randall 1958; Côté 2000; Bshary and Côté 2008). One of the unique characteristics of this system comes from a conflict of interests between partners since cleaners prefer to cheat by eating client mucus, which is detrimental to the client (Grutter and Bshary 2003). Cleaners manage this conflict of interest by adjusting service quality to client identity and situation. For example, they are able to distinguish between predator and non-predator clients as well as between familiar and unfamiliar individuals (Tebich et al. 2002; Bshary et al. 2011), and to behave more cooperatively if bystander clients are present (Bshary and Grutter 2006; Pinto et al. 2011). A widespread ability of cleaners is to largely feed against their preference, so that clients do not terminate interactions prematurely or fail to return for another inspection (Bshary and Grutter 2002, 2005). Thus, it appears that cleaners are able to calculate payoffs without overvaluing current benefits against both immanent and delayed future benefits (Grutter and Bshary 2003).

In laboratory conditions, the associative learning process (food-cue association; Sison and Gerlai 2010) can be investigated by using Plexiglas plates "clients" offering food as proxies for "real clients." This experimental paradigm has been used extensively and successfully in the last few years, both on cleaner wrasses (Bshary and Grutter 2002, 2005, 2006; Grutter and Bshary 2003; Bshary et al. 2008; Danisman et al. 2010; Raihani et al. 2010, 2012a, b, c; Salwiczek and Bshary 2011; Bshary et al. 2011; Gingsins et al. 2013; Wismer et al. 2014; Cardoso et al. 2015) and on other model system of marine cleaning mutualism such as the Caribbean cleaning gobies *Elacatinus* spp. and the saber-toothed blenny *Plagiotremus* spp. (Soares et al. 2010; Bshary and Bshary 2010, 2012). This experimental paradigm captures the essence of cleaning interactions as demonstrations of key results have been reproduced in experiments with real cleaner-client interactions (Pinto et al. 2011). Moreover, Wismer and colleagues (2014) found that cleaner wrasses that lived in a socially simple environment (i.e., small isolated

reefs instead of continuous fringing reefs) failed to learn and perform in ways consistent with previous studies; thus, variation in social context and familiarity with clients may have major relevance to this system. In the current experimental design, we used different color patterns, which allow for individual identification of plates. We mimicked a situation that occurs regularly under natural conditions, namely, when two clients seek service simultaneously (Bshary and Côté 2008). In such situations, cleaners have to make a choice of which client to inspect first, with the risk that the ignored client will leave (Bshary and Schäfer 2002). In these experiments, the ignored plate was always immediately removed by the experimenter. Client species differ with respect to their nutritional value, i.e., parasite load (Grutter 1994; Bansaer et al. 2002; Soares et al. 2008), and we introduced this aspect by offering food on one plate only. Thus, cleaners had to learn to identify the food source.

We conducted two experiments that differed with respect to the relevant cues available to identify the correct choice. In experiment 1, cleaners had to identify the plate that consistently provided food, while in experiment 2, they had to identify a location where food was consistently provided. As cleaners exclusively feed on the surface of clients that are themselves mobile, the first experiment (cue discrimination) is socio-ecologically more relevant while the second experiment (place discrimination) is not. Furthermore, the two different learning tasks presumably rely on different neural substrates, with cue learning being Dm-dependent whereas place learning is Dl-dependent. AVT seems to be a good candidate to affect both types of learning since V1a receptors are widely distributed throughout the forebrain in other teleost species (Huffman et al. 2012). However, variance in the expression of nonapeptide receptors on areas of relevance for learning and memory, such as the Dl and Dm, may allow for differential effects of AVT on Dl- and Dm-dependent learning. Here we predict that, given the general effect of AVT on social behavior and its already established effect on cleaner fish, cooperative behavior in particular, AVT should preferentially alter (i.e., inhibit) the putatively Dm-dependent and ecologically relevant task of cue learning.

Methods

Housing

Experiments were conducted at the fish housing facilities of the Oceanário de Lisboa (Lisbon, Portugal). We used 12 wild caught *L. dimidiatus* that originated in Maldives and were directly imported to Portugal by a local distributor. The fish were kept in individual aquaria (100×40×40 cm) combined in a flow through system that pumped water from a larger cleaning tank (150×50×40 cm) that served as a natural filter. Nitrite concentration was kept to a minimum (always below

0.3 mg/l). Each aquaria system contained air supply and commercial aquarium heaters (125 W, Eheim, Jäger). Small PVC pipes (10–15 cm long, 2.5 cm in diameter) served as shelter for the fish. Each aquarium was divided into two compartments separated by an opaque partition. The partition could be closed or opened by pulling up the partition. Experiments were carried out between February and November 2012 in the individual tanks of each fish. Before the start of the experiments, fish were trained to feed from a Plexiglas plate as a substitute for the client fish that serve as food source in nature. Cleaners took approximately 3 days to learn to eat from plates. We tested the following compound treatments on two learning tasks: saline, AVT (two dosages), Manning compound, and atosiban. Below, we explain it further.

Experiment 1: cue discrimination task

For each treatment, we had two plates (acting as clients) with two different color patterns: One was deemed to be the one from which cleaner needed to start eating first (correct pattern) and the second that could wait but had no food/reward available (incorrect pattern, see Fig. 1). All plates were initially introduced to cleaners, the day before the start of experiments, with a small piece of prawn in the back. Cleaners were submitted to one session, in alternate days. Each session was composed of 10 trials, until the individual had learned (three successive sessions in which the individual chose the correct pattern plate in at least 7 out of 10 trials, two successive sessions in which the cleaner chose the correct pattern plate in at least 8 out of the 10 trials, or just one session with at least 9 out of 10 trials). The first trial began 10 min after the compound treatment and consisted of successive presentation (10-min intertrial interval) of the two pattern color plates until the completion of 10 trials. The “correct” pattern plate had available prawn while the “incorrect” pattern plate had inaccessible prawn (covered with transparent tape which prevented cleaners to smell the item) at the back, so out of sight for cleaners during the choice process. Experimental individuals would then have complete access to both plates; a correct choice would enable both plates to stay inside the aquarium, while an incorrect choice would result in immediate removal of the correct plate, preventing cleaners from eating the food item (Fig. 1). The left–right position of the plates would change randomly among trials.

Experiment 2: place discrimination task

In this task, the cleaner was challenged with learning a specific side of the plate on which to start eating (spatial learning), instead of a correct color pattern plate (cue learning, see Fig 1). Plates were initially introduced to cleaners with a small piece of prawn in the back, one plate at the time, the day before the beginning of experiments. Cleaners were submitted

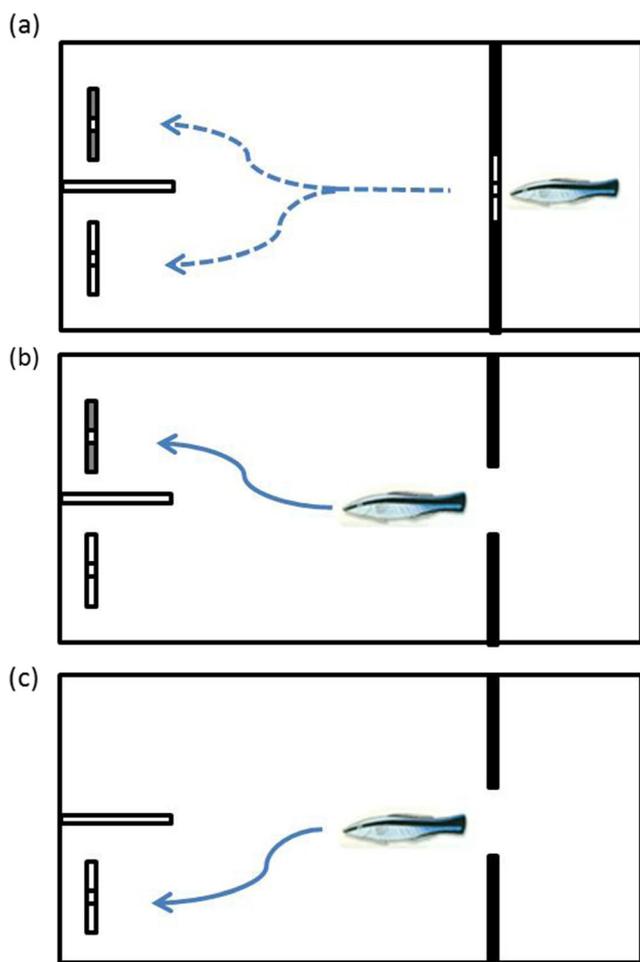


Fig. 1 The experimental aquarium (view from above) was divided into a back compartment (*right*) and the test compartment (*left*) with help of an opaque PVC partition. **a** Initial phase. **b** The two Plexiglas plates are placed, and if the cleaner chooses the correct pattern/side, both plates will stay in the aquarium, with one piece of shrimp available. **c** The two Plexiglas plates are placed, and if the cleaner chooses the incorrect pattern/side, only the incorrect plate remains, with no shrimp available. The two Plexiglas plates were visually separated from each other with help of a dividing transparent partition wall in-between the plates. The tested fish could move into the test compartment when an opaque partition at the side of the back compartment is pulled up

to one session, on alternate days. Each session was composed of 10 trials, until the individual reached our learning criterion as described for the first experiment. The correct side was chosen haphazardly and was maintained between trials and sessions, but it changed between compound treatments. The first trial began 10 min after the injection of the compound treatment and consisted on successive presentation of the two pattern color plates until the outcome of 10 trials. The “correct” side plate had available prawn while the “incorrect” side plate had inaccessible prawn (covered with transparent tape). Experimental individuals would then have complete access to both plates; a correct choice would enable both plates to stay inside the aquarium, while an incorrect choice would consequently have the correct plate (and the only one

with accessible food) immediately removed, preventing cleaners from eating the food item (Fig. 1). The left–right position of the plates would change randomly among sessions.

Neuropeptide treatment

Cleaners were weighed before the onset of the experiment so that injection volume could be adjusted to body weight. All cleaners were used in both experiments. Within each experiment, each cleaner was sequentially and haphazardly injected (intramuscularly) with all the compounds: saline (0.9 NaCl), AVT (V0130–Sigma) at a low (0.5 $\mu\text{g}/\text{gram}$ of body weight (gbw)) and a high (2.5 $\mu\text{g}/\text{gbw}$) dose, Manning compound [3.0 $\mu\text{g}/\text{gbw}$, V2255, Sigma-[b-Mercapto-b,b cyclopentamethylenepropiony]1, O-me-Tyr2, Arg8]-Vasopressin], and atosiban [3.0 $\mu\text{g}/\text{gbw}$, Bachem: H-6722.0050 (Deamino-Cys1, D-Tyr(Et)2, Thr4, Orn8)-Oxytocin(RWJ 22164)]. Injections were always given in the morning (from 8:00 to 12:00). Each individual could in total be injected a maximum of eight times with each compound. However, the real number of injections was usually smaller but dependent on individual learning speed under the effect of each compound. All individuals used were habituated to the injection protocol before the start of experiments as to equalize for the stress of being caught and injected. Manning compound is a commonly used antagonist of the AVP type 1a receptors (V1a), which in teleost fish include both subtypes V1a1 and V1a2, and which has also shown relative affinity for the oxytocin (OT) receptor in mammals (Manning et al. 2008). Atosiban is an antagonist of the oxytocin/isotocin (OT/IT) receptors but has also been referred to have some affinity for AVP/AVT receptors (Manning et al. 2008). If indeed putative AVT effects are being mediated by AVT1a receptors in competition with IT receptors, then the use of both Manning compound and atosiban may help us to interpret their effects more clearly.

Statistical analysis

In both tasks, the same cleaners were used in all treatment groups. Sample size on the place discrimination task is smaller because two of the cleaners were not able to learn the task in the control treatment (saline injection). Data were analyzed using one-way repeated measures (RM) ANOVA to test for main effects of neuropeptide treatment and a posteriori planned comparisons of least squares means in order to contrast the effect of each neuropeptide treatment with the reference (saline) group. All statistical tests shown in this study were two-tailed. Although it would be interesting to compare both tasks, these are not comparable because the second task involves reversal learning (cleaners first learned to make a discrimination, such as choosing the correct color pattern plate and, in the second experiment, they learned to reverse the

choice—i.e., to understand that it was the specific site and not the pattern that was important). Moreover, it would not be correct to compare both directly due to potential effects of experimental order (experiment 2 always followed experiment 1).

Results

Experiment 1: cue discrimination task

We found a significant main effect of treatment on cleaners' ability to solve the cue discrimination problem (RM ANOVA, $F_{4, 44}=7.05$, $p=0.0018$, Fig. 2a). Cleaners injected with Manning compound needed significantly fewer sessions to complete the task than the saline group, whereas those treated with atosiban needed significantly more learning sessions than the saline group to solve the problem (planned comparisons: Manning compound vs saline, $F_{1,11}=12.57$, $p=0.004$; atosiban vs saline, $F_{1,11}=5.18$, $p=0.04$; Fig. 2a). Neither of the AVT dosages had an effect on learning (planned comparisons: AVT 0.5 vs saline, $F_{1,11}=0.41$, $p=0.53$; AVT 2.5 vs saline, $F_{1,11}=3.11$, $p=0.11$, Fig. 2a). A similar pattern was observed in the learning curves for each treatment, where it is clear that the Manning compound learning curve is distinct from the curves of the other treatments (Fig. 3a).

Experiment 2: place discrimination task

We found a marginally non-significant trend for the main effect of treatment on cleaners' ability to learn the place discrimination task (RM ANOVA, $F_{4, 36}=2.38$, $p=0.069$; Fig. 2b). Planned comparisons revealed that cleaners treated with the lower dosage of AVT needed a higher number of sessions to complete the task than fish from the saline treatment (planned comparisons: AVT 0.5 vs saline, $F_{1,9}=10.51$, $p=0.01$; AVT 2.5 vs saline, $F_{1,9}=0.08$, $p=0.78$; Manning compound vs saline, $F_{1,9}=0$, $p=1$; atosiban vs saline, $F_{1,9}=0.07$, $p=0.79$; Fig. 2b). The learning curves were similar among the different treatments, but the learning curve of the lower dosage of AVT was the most distinct from the curve generated by the control treatment (saline) (Fig. 3b).

Discussion

Here we tested AVT's relevance for cleaners' ability to solve two social associative learning tasks that are putatively linked to different neural circuits. Our results show that AVT affects the learning competence of cleaners in both learning tasks, which seem to be related to two parallel memory systems and vary in ecological relevance. In the cue learning task, there was a visual tendency for increasing dosages of AVT

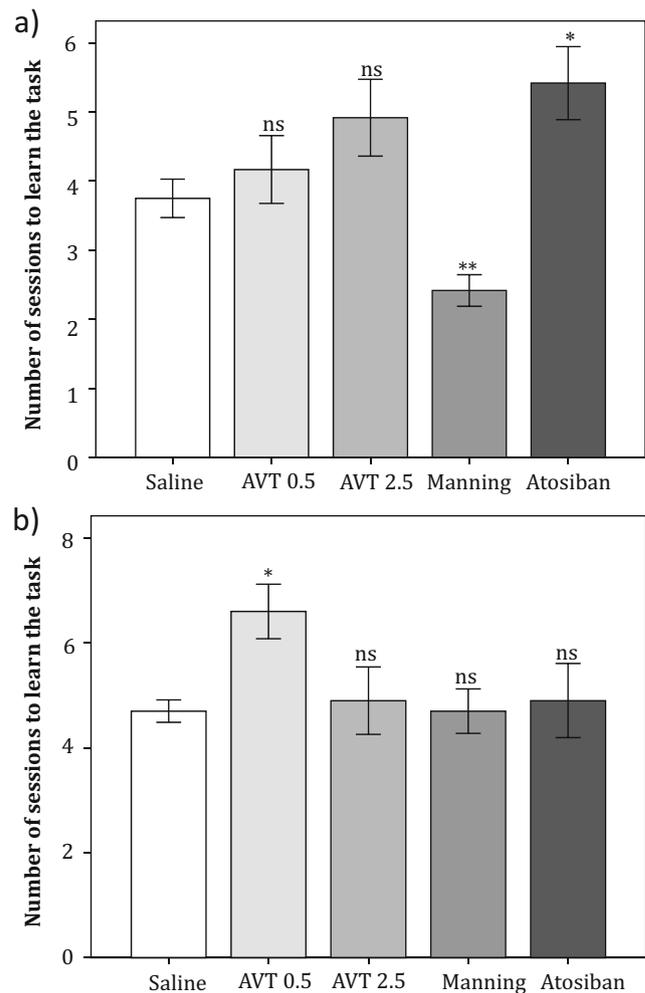
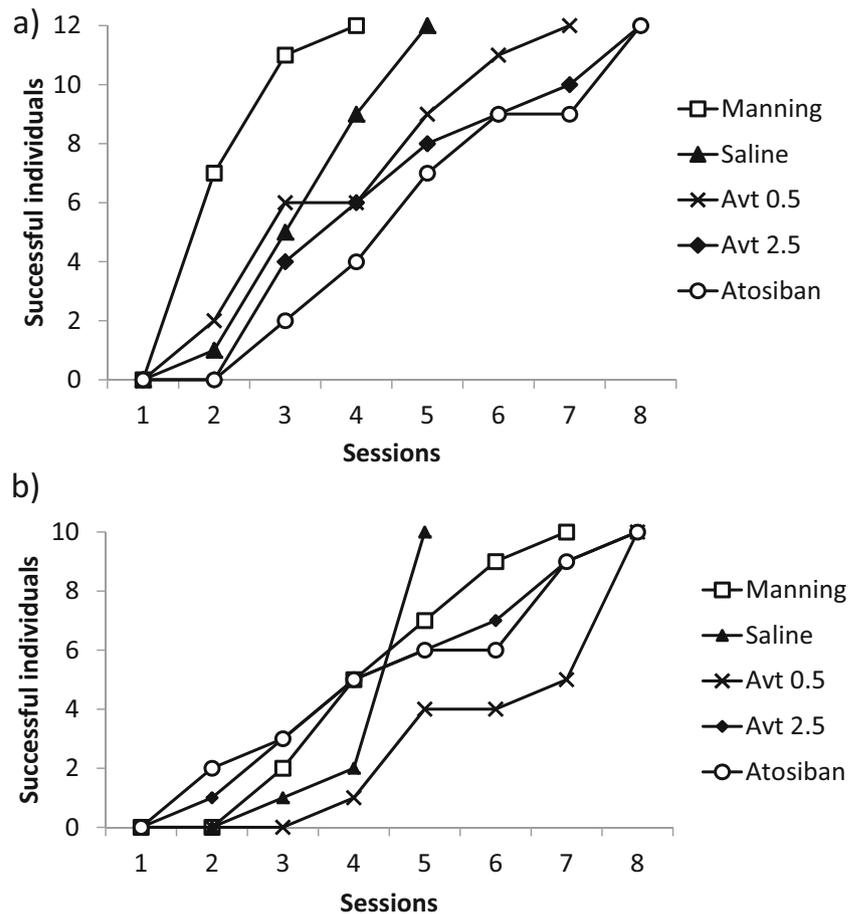


Fig. 2 The effect of the neuropeptides arginine vasotocin (AVT), Manning compound (Manning), and atosiban on learning behavior of the cleaner fish *L. dimidiatus* **a** the ecologically relevant cue-learning task, compared with a control (saline); and **b** the ecologically non-relevant place task, compared with a control (saline). Symbols above bars represent p values which refer to planned comparisons of least squares means effect of each neuropeptide treatment group against the reference (saline) group (* $p<0.05$; ** $p<0.01$; ns >0.05); error bars represent the standard error of the mean. Cleaners had a maximum of eight sessions to learn the task

to delay learning while the AVT antagonist (Manning compound) significantly increased learning speed in relation to the reference treatment (saline injection). In the place learning task, only the small AVT dosage significantly delayed learning speed in comparison to saline-treated fish. Thus, the negative effects of AVT on learning do not seem to be independent of the learning task. Moreover, these effects are associated to the specificity of dosage and to the sensitivity of the tasks regarding AVT. These can be explained by the differential sensitivity of the two neural circuits involved in the two learning types to the different AVT receptors. However, it may also reflect a significant variance in terms of relevance of these circuits on their daily operation: one being extensively used

Fig. 3 Learning curves generated by the different treatments (number of cleaner wrasses that successfully learned the task in each session) in **a** the ecologically relevant cue-learning task and **b** the ecologically non-relevant place task. Cleaners had a maximum of eight sessions to learn the task



when interacting with clients and the other providing some sort of spatial discrimination and territoriality.

Pharmacological manipulations of the AVT system had an effect on both cue and place learning, which is putatively dependent on Dm and Dl, respectively, and suggests that AVT receptors and fiber varicosities might be present in both circuits in cleaner wrasses (as found in other species; Dewan et al. 2011). To date, three AVT receptors subtypes (i.e., V1a1, V1a2, and V2) have been described in teleost fish (Lema 2010; Lema et al. 2012). Moreover, AVT may also bind to the receptors of IT with significant affinity. This is consistent with our results (concerning atosiban's effects), as recent mapping of nonapeptide receptors in the brain of teleost species have shown that V1a and IT receptors are widely distributed throughout the forebrain, being present on both Dm and Dl areas (Kline et al. 2011; Huffman et al. 2012).

The dose-dependent effects described above could be explained by the variable expression of the different receptor subtypes at each memory circuit. For example, in the place learning task, only the lower AVT dosage was effective in slowing down learning, which could mean that at higher AVT concentrations, the binding to other AVT and isotocin receptors (e.g., V1a2), may

compromise the specific effects of binding to a specific AVT receptor (e.g., V1a1). Moreover, the inhibitory effect of AVT in the place learning task may also suggest that spatial representations might also have an influence on cleaning efforts, for instance in the identification of more appropriate or favorite sites to interact with clients or with conspecific partners (within the territorial boundaries). On the other hand, in the cue learning task, the learning enhancing effects found for the V1a antagonist (Manning compound) and the observed non-significant trend for a dose-related detrimental effect of AVT suggests a larger role of AVT and V1a-sensitive circuitry in the mediation of learning in the ecologically relevant task, compared to what was demonstrated in the non-ecologically relevant task.

The differential dose-response results of the two learning tasks to the pharmacological manipulations of the AVT system used in this study may also depend on the binding of AVT to V1a-type or to non-AVT receptors, which may be differently expressed across the two brain memory circuits. The V1a-type receptors (both subtypes V1a1 and V1a2) are generally the most predominant AVT receptors found in the teleost brain

(Kline et al. 2011; Huffman et al. 2012), but these subtypes may not be equally expressed or functionally similar. For example, in the grouper (*Epinephelus adscensionis*), the V1a2 subtype is more expressed in the brain than the V1a1 subtype (Kline et al. 2011), and the expression of the V1a2 subtype is closely linked with sex, reproduction, and behavior, whereas V1a1 receptors in the brain are associated with stress response (Lema et al. 2012). A higher activity of AVT/V1a2 circuitry in the brain has been associated with space use and social dominance, whereas pair bonding seemed to be mediated by a different, less active AVT/V1a2 circuitry (Oldfield et al. 2013). In cleaner wrasses, interspecific cooperative behavior is regulated by the V1a-type brain receptors, which was inferred via pharmacological treatment with the V1a antagonist Manning compound (Soares et al. 2012); however, differences between the effects of both V1a subtypes could not be specified. Nevertheless, the regulation of cooperative behavior should probably rely on shifts at the AVT/V1a2 circuits, a hypothesis that is amenable for further testing. Moreover, it should be noted that the learning-delaying effects of AVT on cue learning in cleaner wrasses contrasts with the memory-enhancing effects of AVP on hippocampus-dependent learning in rodents (Alescio-Lautier and Soumireu-Mourat 1998; Alescio-Lautier et al. 2000). Future work focussing on how AVT regulates the molecular signaling pathways related to learning and memory in cleaner wrasses is needed to further explore these contrasting results.

Exogenously infused levels of AVT have been found to decrease cleaners' likelihood to engage in cleaning interactions with their clients, however, increased their predisposition to interact with conspecific partners (Soares et al. 2012). More recently Cardoso and colleagues (2015a) demonstrated that the administration of AVT leads to a decrease of cleaners' willingness to feed against preference. Hence, in both studies, higher AVT system activity was observed to reduce cleaners propensity to forage but otherwise, seemed to promote a higher motivational state to engage with conspecific partners. Thus, results seem to be consistent with previous studies (e.g., Semsar et al. 2001) suggesting that the AVT system may shift female cleaners' behavior between distinct motivational states, in this case, from maintenance behavior such as foraging to mating behaviour, and vice versa. If higher AVT system activity promotes mating behavior, then it could also shift the motivation away from feeding behavior. In contrary, lower AVT activity may correlate with lower motivation to mate and higher motivation to feed. Thus, when individuals are more motivated to mate (increased AVT activity), they may be less motivated to learn a feeding task, while the converse would also occur, with individuals less motivated to mate (lower AVT system activity) being more motivated to feed,

and in turn more motivated to learn a feeding task. Our cue discrimination results appear to be consistent with this explanation. As place discrimination is deemed to be less ecologically relevant to feeding behavior, it makes sense that blocking AVT activity was unable to produce significant results.

Another potential explanation for the effects of AVT upon cleaner wrasses' behavior is that it could also occur via the activation of a stress response, because AVT influences adrenocorticotropin (ACTH) production and cortisol secretion (Balment et al. 2006), which could in turn prompt an inhibition of learning. Indeed, recent work has shown that exogenous injections of AVT cause a stress response (increased circulation levels of cortisol), which leads to a reduction in behavioral activity (Huffman et al. 2014). However, the effects of increasing cortisol levels in cleaner wrasses seem to differ considerably from other systems. A recent study demonstrated that elevations in cortisol levels are responsible for transient shifts toward a cheating strategy in cleaner fish (Soares et al. 2014). Thus, increasing cortisol levels does not lead to a reduction in activity levels but it rather seems to be associated with specific behavioral changes in the cleaner wrasse system. It is thus difficult to directly associate the effects of AVT's learning inhibition reported here to the potential rise of circulating cortisol.

Previous evidence on the cleaner wrasse system has demonstrated that cleaners can recognize individual clients (Tebbich et al. 2002) and are able adjust to past experience with them (Bshary and Grutter 2002, 2005). This means that cleaners' cooperative life comes with specialized cognitive skills that include social recognition and associative learning, which might be presumably mediated by AVT and IT (Soares et al. 2012; Cardoso et al. 2015a, b). Overall, our findings demonstrate that AVT/V1a pathways, which are implicated in the regulation of interspecific behavior, i.e., a cleaner's willingness to seek interactions with clients, are also linked to individual learning ability, in the context of their mutualistic behavior, and in tune with their socio-ecological demands. Future work should focus further on AVT's influence in learning by comparing its role in tasks that are more strongly associated with feeding with tasks that are associated with mating behaviors; for example, feeding alone versus feeding in the context of mixed-sex pairs.

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Author contributions MCS and RB designed the study. SCC, JRP, and RM collected the data. SCC, MSC, RM, RFO, and RB analyzed the data and wrote the paper. All authors discussed results and commented on the manuscript.

Compliance with Ethical Standards Animal procedures used in this study have been approved by the Portuguese Veterinary Office (Direcção Geral de Veterinária, license # 0420/000/000/2009).

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