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RESEARCH ARTICLE

Effects of neurotransmitter receptor antagonists on sea urchin righting behavior and tube foot motility

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ABSTRACT

Echinoderms, such as sea urchins, occupy an interesting position in animal phylogeny in that they are genetically closer to vertebrates than the vast majority of all other invertebrates but have a nervous system that lacks a brain or brain-like structure. Despite this, very little is known about the neurobiology of the adult sea urchin, and how the nervous system is utilized to produce behavior. Here, we investigated effects on the righting response of antagonists of ionotropic receptors for the neurotransmitters acetylcholine, GABA and glycine, and antagonists of metabotropic receptors for the amines dopamine and noradrenaline (norepinephrine). Antagonists slowed the righting response in a dose-dependent manner, with a rank order of potency of strychnine>haloperidol>propranolol>bicuculline>hexamethonium, with RT50 values (concentrations that slowed righting time by 50%) ranging from 4.3 µmol l^{-1} for strychnine to 7.8 mmol l^{-1} for hexamethonium. The results also showed that both glycine and adrenergic receptors are needed for actual tube foot movement, and this may explain the slowed righting seen when these receptors were inhibited. Conversely, inhibition of dopamine receptors slowed the righting response but had no effect on tube foot motility, indicating that these receptors play roles in the neural processing involved in the righting behavior, rather than the actual physical righting. Our results identify the first effects of inhibiting the glycinergic, dopaminergic and adrenergic neurotransmitter systems in adult sea urchins and distinguish between the ability of sea urchins to right themselves and their ability to move their tube feet.

KEY WORDS: Echinoderm, Glycine receptor, nAChR, GABA receptor, Dopamine receptor, Adrenergic receptor, *Strongylocentrotus purpuratus*

INTRODUCTION

Echinoderms, particularly sea urchins, are an important model organism in developmental biology (Adonin et al., 2021). However, vey little is known about the neurobiology of adult sea urchins following their metamorphosis from bilateral larvae to pentaradial adult animals or how this compares with the neurobiology of non-deuterostome invertebrates, and with that of fellow deuterostomes, the vertebrates. Sea urchins lack a brain structure but they do have a central nervous system consisting of a central nerve ring encircling the Aristotle's lantern from which project five radial nerves, which in turn innervate the hundreds of tube feet and spines, and small ganglia of the peripheral nervous system (Burke et al., 2006; Mashanov et al., 2016). The tube feet and spines are used in movement and as sensory organs (Reese, 1966). The first echinoderm genome to be sequenced was that of the purple sea urchin, *Strongylocentrotus purpuratus* (Sea Urchin Genome Sequencing Consortium, 2006), and it revealed the presence of genes encoding putative receptors for the ubiquitous neurotransmitters acetylcholine, GABA, glycine, serotonin, dopamine and noradrenaline (norepinephrine) (Burke et al., 2006). Given the relatively simple anatomical organization of the sea urchin nervous system compared with that of other deuterostomes, and the availability of genomic data, the adult sea urchin would seem to be an ideal organism to study the molecular mechanisms of behavior in an animal that bridges the divide between more well-studied invertebrate phyla such as Arthropoda and Nematoda, and the vertebrate chordates.

There is substantial evidence that adult sea urchin tissues contain acetylcholine, GABA, serotonin and dopamine (Pentreath and Cobb, 1972; Bachmann and Goldschmid, 1978). Despite the presence of neurotransmitters and neurotransmitter receptor genes, there is little functional information on the identity of the major neurotransmitters utilized by adult echinoderms in specific behaviors. Of the major neurotransmitters, only acetylcholine has been investigated in depth in terms of function in adult sea urchins. Acetylcholine, via excitatory nicotinic acetylcholine receptors (nAChRs), has been demonstrated to elicit contraction of isolated tube feet (Florey et al., 1975; Florey and Cahill, 1980), radial muscle (Tsuchiya and Amemiya, 1977) and lantern retractor muscle (Boltt and Ewer, 1963; Kobzar and Shelkovnikov, 1985), and nAChR activation modulates the mechanical properties of sea urchin ligaments (Morales et al., 1989, 1993; Birenheide et al., 1996; Wilkie et al., 2015). Contrary to its more usual role as an inhibitory neurotransmitter, GABA has been shown to have an excitatory effect on sea urchin tube foot contraction (Florey et al., 1975), and it was concluded that the GABA receptors were neuronal rather than being colocalized with post-synaptic nAChRs on tube foot muscle. In other echinoderms, GABA has been shown to be excitatory in isolated tube feet of *Strongylocentrotus franciscanus*, *Arbacia* and *Echinus* sea urchins (Florey et al., 1975), as well as in *Asterias* sea star tube feet (Protas and Muske, 1980). There is also evidence of an inhibitory action of GABA in *Isostichopus* and *Sclerodactyla* sea cucumbers (Devlin, 2001) and in the retractor muscle of *Parechinus* sea urchins (Boltt and Ewer, 1963).

Evidence for dopamine and adrenergic receptor function in adult sea urchins is also sparse but both have been shown to modulate the mechanical properties of ligaments in sea urchins (Morales et al., 1993). In other echinoderms, dopamine and noradrenaline have been detected in regenerating sea star arms (Huet and Franquinet, 1981) and, along with serotonin, can inhibit acetylcholine-induced luminescence of sea star photocytes (De Bremaeker et al., 2000). Serotonin can also inhibit acetylcholine-induced contractions of

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Received 24 June 2021; Accepted 22 February 2022

Journal of Experimental Biology (2022) 225, jeb243076. doi:10.1242/jeb.243076

body wall muscles in sea cucumbers (Inoue et al., 2002), and both serotonin and dopamine are present in the radial nerve cord of sea cucumbers (Chaiyamoon et al., 2018). To date, no studies have examined the roles of serotonin receptors in adult sea urchins or of glycine receptors in any echinoderms.

In addition to classical neurotransmitters, many neuropeptide genes and gene products have been identified in sea urchins (Burke et al., 2006; Rowe and Elphick, 2012; Monroe et al., 2018) and some have been shown to regulate muscle contraction in sea urchins and other echinoderms (Elphick, 2014). Finally, the gaseous neurotransmitter nitric oxide has been shown to play a role in muscle relaxation in sea urchin tube feet (Billack et al., 1998) and in sea stars (Elphick and Melarange, 2001), and inhibition of nitric oxide synthase can inhibit the sea urchin righting response (Shah et al., 2018).

The roles of specific neurotransmitters in sea urchin neurobiology have therefore not been extensively studied or, in some cases, are completely unknown. In particular, the functional roles, or lack of roles, of different neurotransmitters in normal sea urchin behavior have not been determined. To investigate neurochemical mechanisms underlying behavior, we utilized the righting response. The sea urchin righting response occurs when an inverted sea urchin utilizes its tube feet and spines to right itself. The response itself occurs as a result of a lack of contact between its oral surface and the substrate, rather than any absolute requirement to be orientated with its oral surface facing downwards (Parker, 1922). Using the time to right, we quantified the effects of well-characterized neurotransmitter receptor antagonists on the righting response as well as on the overall motility of the sea urchin's tube feet. This allowed us to examine whether the observed effects on righting were due to effects on neural processing or changes in the ability of actual tube feet to move and be utilized in the righting response.

MATERIALS AND METHODS

Animals

Purple sea urchins, *Strongylocentrotus purpuratus* (Stimpson 1857), approximately 60 mm in test diameter, from the Pacific Ocean were obtained from Marinus Scientific (Long Beach, CA, USA) and maintained in aerated tanks at 13°C containing artificial seawater (ASW) (Instant Ocean, That Pet Place, Lancaster, PA, USA) made according to the manufacturer's instructions. All behavioral tests were conducted in ASW solutions chilled to 10–15°C.

Drug administration

All drugs were purchased from Millipore Sigma (St Louis, MO, USA). The following drug stock solutions were made and used immediately or stored at −20°C until required: 100 mmol l^{-1} hexamethonium in ASW, 100 mmol l^{-1} bicuculline in DMSO, 10 mmol l^{-1} strychnine in ethanol, 100 mmol l^{-1} haloperidol in methanol and 100 mmol l^{-1} propranolol in DMSO. Test solutions were made fresh each day from stocks and diluted in ASW. To administer the drugs, sea urchins were immersed in tanks containing ASW and the specified drug for 1 h and the behavioral tests were conducted in the drug solutions.

Behavioral assays

To test the effect of the drugs on righting, a righting assay was used. Sea urchins were inverted and placed on their aboral side on the base of a tank containing ASW with or without the specified drugs, and the time to completely right themselves was recorded. Healthy, untreated sea urchins typically right themselves in around 3 min (Shah et al., 2018). A cut off of 15 min was applied to the righting experiments, and for statistical analysis, this cut-off value was used as the time to right. If sea urchins in control conditions failed to right within 15 min, they were excluded from further testing. Variable temperature righting experiments and tube foot motility experiments were conducted over the range of 10–15°C, monitored

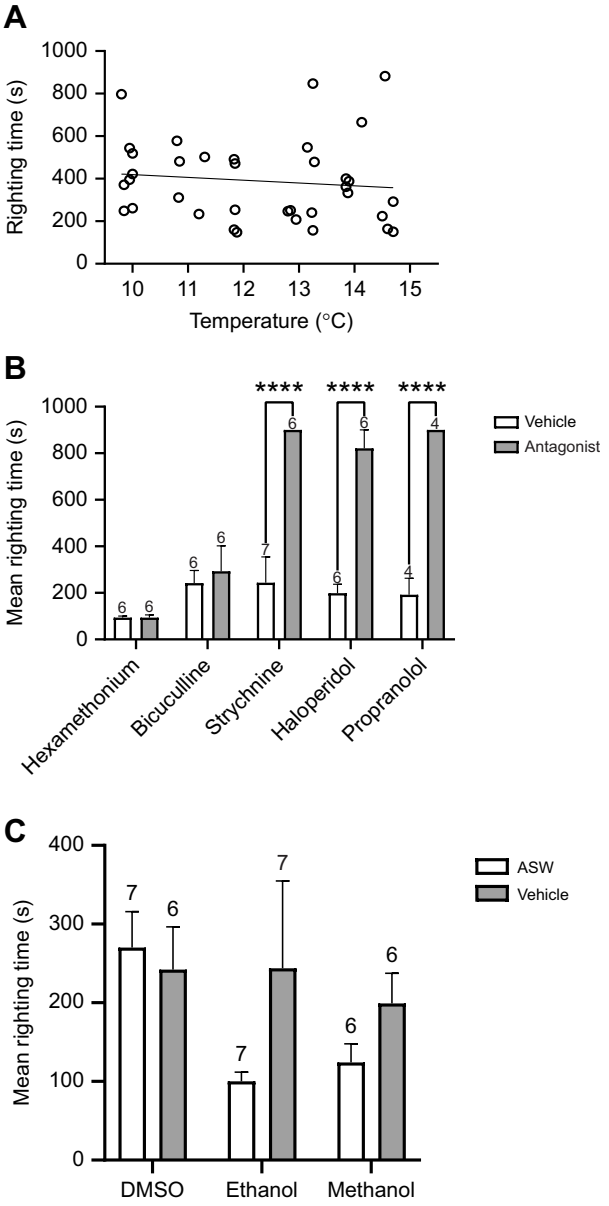

Fig. 1. Mean righting time of *Strongylocentrotus purpuratus* in the presence and absence of neurotransmitter receptor antagonists. (A) The righting time of individual urchins was measured over the indicated temperature range, which was found to have no significant effect (R=0.01, P=0.51, N=36). (B) Mean (±s.e.m.) righting time was measured for sea urchins in the presence of vehicle or 100 μmol l^{-1} neurotransmitter receptor antagonist. (C) Mean (±s.e.m.) righting time was also measured for sea urchins in artificial seawater (ASW) and in ASW plus the relevant vehicle. Numbers above bars indicate the number of sea urchins. Bicuculline and propranolol were dissolved in DMSO, strychnine was dissolved in ethanol, and haloperidol was dissolved in methanol; no organic solvent was needed to dissolve hexamethonium. Significant differences are indicated with asterisks (****P<0.0001).

with a thermocouple digital thermometer (Minnesota Measurement Instruments, St Paul, MN, USA).

An assay was developed to quantify the movement of sea urchin tube feet. A single sea urchin was immersed in ASW right-side up (with or without drug) in a circular tank (12.7 cm diameter) atop a printed template composed of a circle divided into 16 equally sized segments. One-minute recordings (Olympus Tough TG-5 camera, 8.3 MP, 30 frames s^{-1}) were obtained from directly above the sea urchin at a distance of 28 cm. Recordings were renamed with random numerical file names to blind the user to the identity of the experimental conditions and analyzed in BORIS software (Friard and Gamba, 2016) by focusing on a single segment line for the duration of the recording, and each time any tube foot crossed this line segment, an event was recorded. This was then repeated for all other line segments, counting every time each tube foot crossed a line segment, and the events were summed to give the total number of events for a single urchin under a single condition.

Dose–response data were fitted using the equation:

$$y = \min + \left(\frac{x^h(\max - \min)}{x^h + \mathrm{RT50}^h}\right), \quad (1)$$

where RT50 represents the drug concentration at which 50% of the maximum slowing of righting time was observed, and min and max are the minimum and maximum righting times, respectively. All *t*-tests are two-tailed paired tests and assumed non-equivalent variance. Multiple comparisons between control and drug conditions were analyzed using two-way ANOVA tests with Bonferroni correction. Error bars indicate s.e.m. Differences were deemed significant at the $P<0.05$ level.

RESULTS

To determine whether specific neurotransmitters play roles in sea urchin behavior, we sought to determine whether the inhibition of major neurotransmitter systems could inhibit or slow the righting response, in which an inverted sea urchin reorients itself, a response that necessarily involves multiple sensory and motor systems, with the aim of determining whether a given neurotransmitter system is required for righting. Control righting experiments were conducted within a temperature window of 10–15°C, and an overall mean righting time of 387±33 s (N=35) was recorded; there was also no significant effect of temperature on the righting time over this temperature range (Fig. 1A).

To determine whether different neurotransmitters had any effect on righting time, we initially tested different neurotransmitter receptor antagonists at a concentration of 100 µmol l^{-1} and found that application of the ionotropic acetylcholine receptor antagonist hexamethonium and the ionotropic GABA receptor antagonist bicuculline did not significantly slow the righting response (P=1.0 for both antagonists), whereas strychnine, haloperidol and propranolol, antagonists of glycine receptors, dopamine receptors and adrenergic receptors, respectively, all significantly reduced the righting time ($P<0.0001$ in all cases) compared with vehicle alone (Fig. 1B and Table 1). To control for any potential effect on righting time of the various different solvents used, righting experiments were conducted in control ASW and the specified solvents at the appropriate final concentrations, in the absence of neurotransmitter receptor antagonists. No significant effects on the time to right were found for any of the solvents (1% DMSO, P=1.0; 1% ethanol, P=0.26; 0.1% methanol, P=1.0; two-way ANOVA with Bonferroni's correction) (Fig. 1C). Taken together, these data indicate a role for the glycinergic, dopaminergic and adrenergic systems in the righting response.

Previous work using hexamethonium has indicated a role for nAChRs in muscle contraction (Florey et al., 1975) and in the righting response, albeit at high concentrations (Shah et al., 2018). We thus repeated the righting experiments at a range of drug concentrations to obtain dose–response relationships for the inhibitory effect of the different neurotransmitter systems on righting. At high enough concentrations, all of the antagonists slowed the righting response and the rank order of potency of the inhibitors was strychnine>haloperidol>propranolol>bicuculline>hexamethonium, with hexamethonium only being able to inhibit the righting response at millimolar concentrations (Fig. 2). Interestingly, although at the highest concentrations of each drug tested, righting could be substantially slowed or abolished, this seemed to have no permanent ill effect on the sea urchins. Within a few hours of removal from the drug solution, all of the sea urchins regained use of their tube feet, moved around their holding tanks and resumed feeding behavior.

Given that the drugs used were applied to the ASW and would need to penetrate the sea urchin's tissues to exert their actions, it is reasonable to suspect that hydrophobicity of the compounds may affect their potency. A log–log plot of hydrophobicity (log$P$) against the determined RT50 values (Fig. 3) showed no significant correlation between these parameters (R^2=0.19, P=0.46), suggesting that the differing potencies observed are not the result of the differing abilities of the drugs to be absorbed and distributed in the sea urchin tissue.

Although 100 µmol l^{-1} bicuculline did not significantly slow the righting response, at this concentration the tube feet appeared to be visibly hyperactive. Conversely, at high concentrations of hexamethonium, the tube feet are visibly flaccid (Shah et al., 2018). Given that GABA receptors are typically inhibitory (but see

Table 1. Mean righting time of sea urchins exposed to neurotransmitter receptor antagonists

Receptor	Condition	Righting time (s)	95% CI (s)	*N*	*P*
nAChR	ASW	93.5±7.07	76–111	6	
	Hexamethonium in ASW	93.8±11.7	65–122	6	1.0
$GABA_A$	0.1% DMSO	242±54.4	109–375	6	
	Bicuculline in 0.1% DMSO	293±110	39–547	8	1.0
Glycine	1% Ethanol	244±111	−19–507	7	
	Strychnine in 1% ethanol	900±0.00	900[a]	6	<0.0001
Dopamine	0.1% Methanol	199±38.3	105–293	6	
	Haloperidol in 0.1% methanol	821±78.8	628–1014	6	<0.0001
Noradrenaline	ASW	192±71.0	−5–389	4	
	Propranolol in ASW	900±0.00	900[a]	4	<0.0001

Righting time (means±s.e.m., with 95% confidence interval, CI) was measured in sea urchins exposed to 100 µmol l^{-1} of receptor antagonist in the relevant vehicle or to vehicle alone. nAChR, nicotinic acetylcholine receptor. *P*-values refer to the drug plus vehicle compared with the vehicle alone, compared across all conditions using a two-way ANOVA with Bonferroni correction. [a]In these cases, all of the sea urchins in the experimental group failed to right within the 900 s time limit.

Journal of Experimental Biology (2022) 225, jeb243076. doi:10.1242/jeb.243076

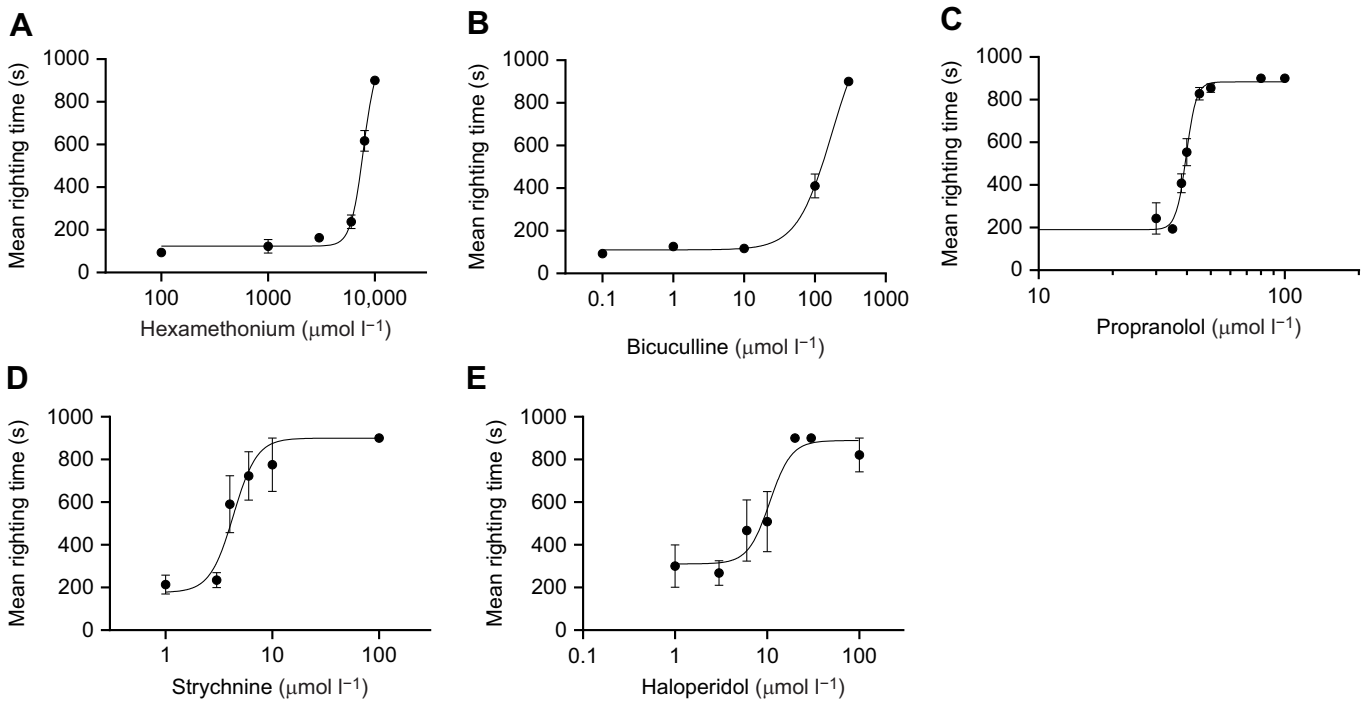

Fig. 2. Dose–response relationships of mean righting time in the presence of different neurotransmitter receptor antagonists. Mean (±s.e.m.) righting time in the presence of (A) the nicotinic acetylcholine receptor (nAChR) antagonist hexamethonium (concentration that slowed righting time by 50%, RT50=7.8 mmol l^{-1}; Hill slope=7.3), (B) the $GABA_A$ receptor antagonist bicuculline (RT50=173 µmol l^{-1}, Hill slope=1.8), (C) the adrenergic receptor antagonist propranolol (RT50=39.8 µmol l^{-1}, Hill slope=21.0), (D) the glycine receptor antagonist strychnine (RT50=4.31 µmol l^{-1}, Hill slope=3.6) and (E) the dopamine receptor antagonist haloperidol (RT50=10.7 µmol l^{-1}, Hill slope=3.7). In some cases, error bars are smaller than data symbols. *N*=4–7 for each data point.

Florey et al., 1975), and that nAChRs are excitatory, it may be that the opposing actions of these two systems influence righting behavior by modulating the motility of the tube feet. We therefore developed a quantitative assay to measure the motility of tube feet, independent of the righting response (Fig. 4A). Control experiments were conducted within a temperature window of 10–15°C, to determine whether this temperature range had any effect on tube foot motility. Overall mean tube foot motility across this temperature range was 399±24 (*n*=30) and there was also no significant effect of temperature on tube foot motility over this temperature range (Fig. 4B).

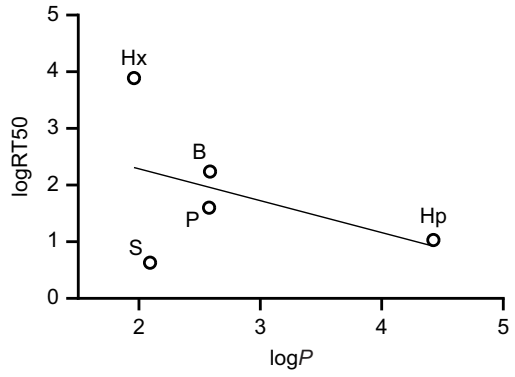

Fig. 3. Relationship between the hydrophobicity of neurotransmitter receptor antagonists and measured RT50 values for righting. The log–log plot of hydrophobicity (log*P*) against the determined RT50 was fitted with a straight line. There was no significant correlation between these parameters. Hx, hexamethonium; S, strychnine; B, bicuculline; P, propranolol; Hp, haloperidol.

Neither 100 µmol l^{-1} hexamethonium (*P*=1.0) nor 100 µmol l^{-1} bicuculline (*P*=1.0) significantly altered tube foot motility compared with vehicle alone (Fig. 4C). Even at 300 µmol l^{-1}, bicuculline did not affect tube foot motility compared with vehicle alone (*P*=0.334, vehicle: mean±s.e.m. 282±33, 95% CI 197–367; vehicle plus bicuculline: 225±62, 95% CI 66–384, *n*=5 for both conditions). We therefore hypothesized that other major neurotransmitters must be responsible for the motility of the tube feet. Strychnine (*P*=0.023) and propranolol (*P*=0.046) both significantly reduced tube foot motility, whereas haloperidol did not (*P*=0.404), compared with vehicle alone (Fig. 4C, Table 2). As with the righting experiments, motility measurements conducted in vehicle alone versus ASW demonstrated no significant effect on motility (DMSO, *P*=1.0; ethanol, *P*=0.18; methanol, *P*=0.79; two-way ANOVA with Bonferroni's correction) (Fig. 4B). Thus, the adrenergic and glycinergic systems are needed for normal motility and, surprisingly, motility could be inhibited by the normally inhibitory glycinergic neurotransmitter system.

DISCUSSION

Our results provide the first example of a role for glycine, dopamine and noradrenaline in the neurobiology of the adult sea urchin, and indicate that all three of these neurotransmitter types are involved in neural control of the righting response in sea urchins. The behavior of righting is a complex series of sensory, neural and neuromuscular steps. The inverted orientation must be sensed, appropriate processing performed in the nervous system to coordinate a response, and coordinated instructions sent to a subset of the large number of tube feet and spines to right the sea urchin. It may be that these neurotransmitter systems are required for all or only a subset of these processes. Our results from the tube foot motility assays enabled us to determine whether each of these systems were

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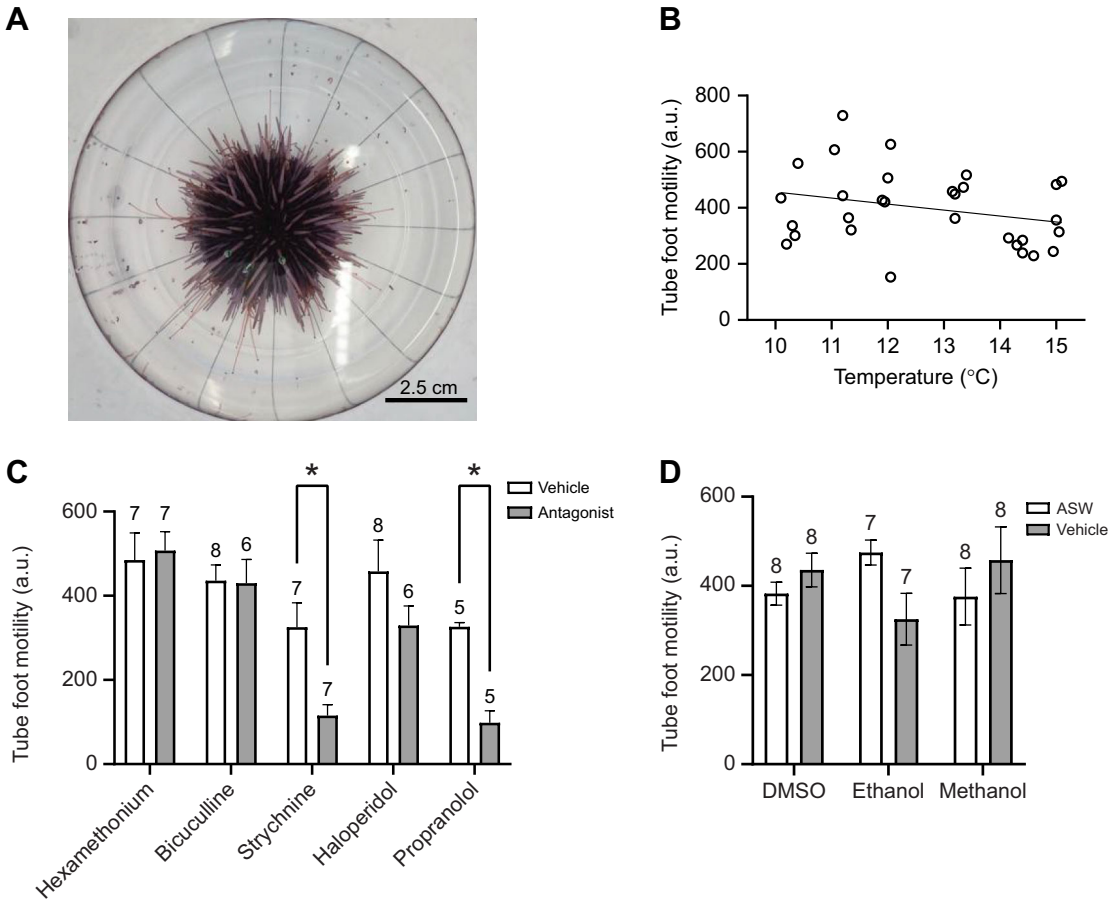

Fig. 4. Mean tube foot motility of *S. purpuratus* in the presence of neurotransmitter receptor antagonists. (A) Tube foot motility was quantified by logging the number of instances that any tube foot crossed into any other segment of a template placed below the testing tank (see Materials and Methods; Movie 1). (B) The tube foot motility score (arbitrary units, a.u.) was measured over the indicated temperature range, which was found to have no significant effect on tube foot motility (R=0.08, P=0.14, N=30). (C) Mean (±s.e.m.) sea urchin tube foot motility was measured in the presence of vehicle or 100 μmol l^{-1} neurotransmitter receptor antagonist. (D) Mean (±s.e.m.) sea urchin tube foot motility was also measured in ASW and in ASW plus the relevant vehicle. Numbers above bars indicate number of sea urchins. Bicuculline and propranolol were dissolved in DMSO, strychnine was dissolved in ethanol, and haloperidol was dissolved in methanol; no organic solvent was needed to dissolve hexamethonium. Significant differences are indicated with an asterisk (*P<0.05).

involved in the process of actual tube foot movement, separate from the actions of sensing righting, and coordinating the response.

The role of glycine receptors

Inhibition of glycine receptors reduced tube foot motility, and also slowed the righting response, indicating an overall excitatory role for glycine in tube foot movement. Glycine receptors in vertebrates are Cl^- permeable and, in most circumstances, their activation results in an inhibition of cell excitability as a result of Cl^- influx. Interestingly, there is also evidence that the activation of the typically inhibitory GABA receptors also results in muscle excitation (Florey et al., 1975). This may suggest that Cl^- flux in adult urchins is actually an efflux, as this would result in cell membrane depolarization (although this does not completely rule out potential effects of excitatory metabotropic GABA receptors). Such a phenomenon is seen in the early development of the nervous system (Ito, 2016) as a result of high intracellular chloride concentrations rendering glycine and GABA ionotropic receptors excitatory. Alternatively, it may be that at the receptor level the glycine receptors are inhibitory, but at the circuit level the glycinergic neurons may be acting to inhibit an inhibitory pathway in motor activity, and therefore pharmacological block of the glycine receptors would result in increased inhibition and reduced motor activity. The *S. purpuratus* genome contains two genes for glycine receptor subunits (Burke et al., 2006) and it will be informative to determine in future work where specifically the glycine receptors are expressed.

The role of adrenergic receptors

Inhibition of adrenergic receptors with propranolol slowed the righting response and reduced tube foot motility, similar to the inhibition of glycine receptors. Adrenergic receptors can in principle be activated by either noradrenaline or adrenaline, both of which are ultimately produced from dopamine. Modification of dopamine by the dopamine β-hydroxylase (DBH) produces noradrenaline and in vertebrates a further enzyme, phenylethanolamine *N*-methyltransferase (PNMT) modifies noradrenaline to produce adrenaline. Analysis of the genome of *S. purpuratus* has revealed a DBH-like gene but no PNMT-like genes, suggesting that sea urchins rely solely on noradrenaline for adrenergic signaling (Burke et al., 2006). The simplicity of the *S. purpuratus* adrenergic system, compared with that in vertebrates, is further demonstrated by the presence of a single adrenergic receptor gene in the *S. purpuratus* genome (Burke et al., 2006). In vertebrates, noradrenaline has roles

Table 2. Mean tube foot motility score of sea urchins exposed to neurotransmitter receptor antagonists

Receptor	Condition	Motility (a.u.)	95% CI (a.u.)	*N*	*P*
nAChR	ASW	485±64.4	333–637	7	
	Hexamethonium in ASW	507±44.6	402–612	7	1.0
$GABA_A$	0.1% DMSO	436±37.6	349–523	8	
	Bicuculline in 0.1% DMSO	430±56.7	291–569	6	1.0
Glycine	1% Ethanol	325±58.0	188–462	7	
	Strychnine in 1% ethanol	115±25.9	54–176	7	0.023
Dopamine	0.1% Methanol	458±74.9	285–631	8	
	Haloperidol in 0.1% methanol	329±46.4	215–443	6	0.404
Noradrenaline	ASW	326±10.1	300–352	5	
	Propranolol in ASW	98.4±28.2	26–171	5	0.046

Tube foot motility scores (a.u., arbitrary units; means±s.e.m., with 95% confidence interval, CI) of sea urchins exposed to 100 µmol l^{-1} of receptor antagonist in the relevant vehicle or to vehicle alone. nAChR, nicotinic acetylcholine receptor. *P*-values refer to the drug plus vehicle compared with the vehicle alone, compared across all conditions using a two-way ANOVA with Bonferroni correction.

in alertness, memory, attention, heart rate and regulation of blood pressure (Docherty, 2019), where the noradrenaline-mediated contraction of vertebrate smooth muscle cells lining blood vessels increases blood pressure. Echinoderms lack a blood vascular system, but they do have a water vascular system that is responsible for the movement of tube feet and this water vascular system may be regulated in a similar manner to the vascular smooth muscle systems of vertebrates.

The role of dopamine receptors

Inhibiting dopamine receptors slowed the righting response but had no effect on tube foot motility, suggesting that dopamine plays a role in the early steps in the process of righting, such as sensing the inverted orientation, committing to righting or the coordination of tube foot movement, but has no role in the actual movement of the tube feet. Dopamine has long been recognized as having a role in motor control, motivation and reward in other animals (Wise, 2004) and it may be playing a similar role in sea urchins. The *S. purpuratus* genome contains seven genes for dopamine receptors (Burke et al., 2006) and it will be interesting in future work to determine exactly which cells these receptors are localized to. It remains to be determined whether inhibition of the dopaminergic system prevents sea urchins from righting as a result of defects in sensing inversion or being able to coordinate a response, or whether it removes the motivation to right.

The role of nAChRs and GABA receptors

Inhibition of both nAChRs and GABA receptors slowed the righting time, albeit at higher inhibitor concentrations than for the other tested neurotransmitter receptors (RT50 of 7.8 mmol l^{-1} and 173 µmol l^{-1}, respectively). There is a large amount of evidence that sea urchin tube foot muscles utilize acetylcholine (Florey et al., 1975; Florey and Cahill, 1980; Billack et al., 1998; Devlin, 2001) and at millimolar concentrations of the nAChR inhibitor hexamethonium, sea urchin tube feet are visibly flaccid (Shah et al., 2018). The high RT50 value measured for hexamethonium may be in part due to the presumably low absorption rate of the doubly charged hexamethonium molecules.

As with dopamine receptor inhibition, albeit at higher antagonist concentrations, inhibition of GABA receptors also slowed righting, but had no effect on tube foot motility, suggesting a role for GABA in central processing rather than a direct action on tube foot movement. Recent work on the sea urchin *Hemicentrotus pulcherrimus* has demonstrated that GABA is needed for the creeping movement of metamorphic juveniles and that GABA has an excitatory role not only in sea urchins (Katow et al., 2020) but also in sea stars (Protas and Muske, 1980), but an inhibitory role in sea cucumbers (Devlin, 2001). As with our results on glycine receptors, it is unclear whether the excitatory action of $GABA_A$ receptors seen in sea urchin righting results from Cl^- efflux from cells, or whether the receptors themselves are actually inhibitory and the inhibition arises at the circuit level.

Conclusions

Although multiple studies have determined the presence of catecholamines and their roles in the development and physiology a of sea urchin larvae (Burke, 1983; Gustafson, 1991; Wada et al., 1997; Katow et al., 2010), and catecholamines have been detected in adult sea urchins (Pentreath and Cobb, 1972; Bachmann and Goldschmid, 1978; Morales et al., 1993), this is the first study to determine functional roles for catecholamines in adult sea urchin behavior. Our results suggest that noradrenaline plays a direct role in tube foot movement to enable the righting response, whereas dopamine has a role in the righting response distinct from the actual movement of tube feet. It would be of interest to determine the location of these neurotransmitter receptors in the neuronal circuitry of the adult sea urchin and, in the case of dopamine, to determine whether it plays roles in other sea urchin behaviors, which may indicate a general role in motivation in sea urchins or the coordination of tube foot activity. Additionally, to our knowledge, these studies provide the first evidence of functional glycinergic neurotransmission not only in sea urchins but also in a non-chordate deuterostome. For many years, glycine receptors were believed to be restricted to vertebrates. However, more recent work has demonstrated functional glycine receptors in the tunicate chordate *Ciona intestinalis* (Nishino et al., 2010) and at the other end of the invertebrate phylogenetic tree, the primitive invertebrate *Hydra* (Ruggieri et al., 2004). Characterization of the sea urchin glycine receptor may shed light on the evolution of glycine-gated pentameric ligand-gated ion channels in deuterostomes.

Acknowledgements

We thank Madeleine Rumingan, Peri Prestwood, Grace Donner, Mac Gortney and Madison Reid for the care and maintenance of the sea urchins during this project.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: N.P.G., C.S.; Methodology: M.M., N.P.G., C.S.; Validation: C.S.; Formal analysis: M.M., N.P.G., E.H., D.L., S.H., P.R., A.L., F.U., J.B., C.S.; Investigation: M.M., N.P.G., E.H., D.L., S.H., P.R., A.L., C.S.; Resources: C.S.; Writing - original draft: C.S.; Visualization: C.S.; Supervision: C.S.; Project administration: C.S.; Funding acquisition: C.S.

Funding
This work was supported by the University of the South Conduff and Kresge funds and a University of the South Sewanee Undergraduate Research Fellowship awarded to E.H.

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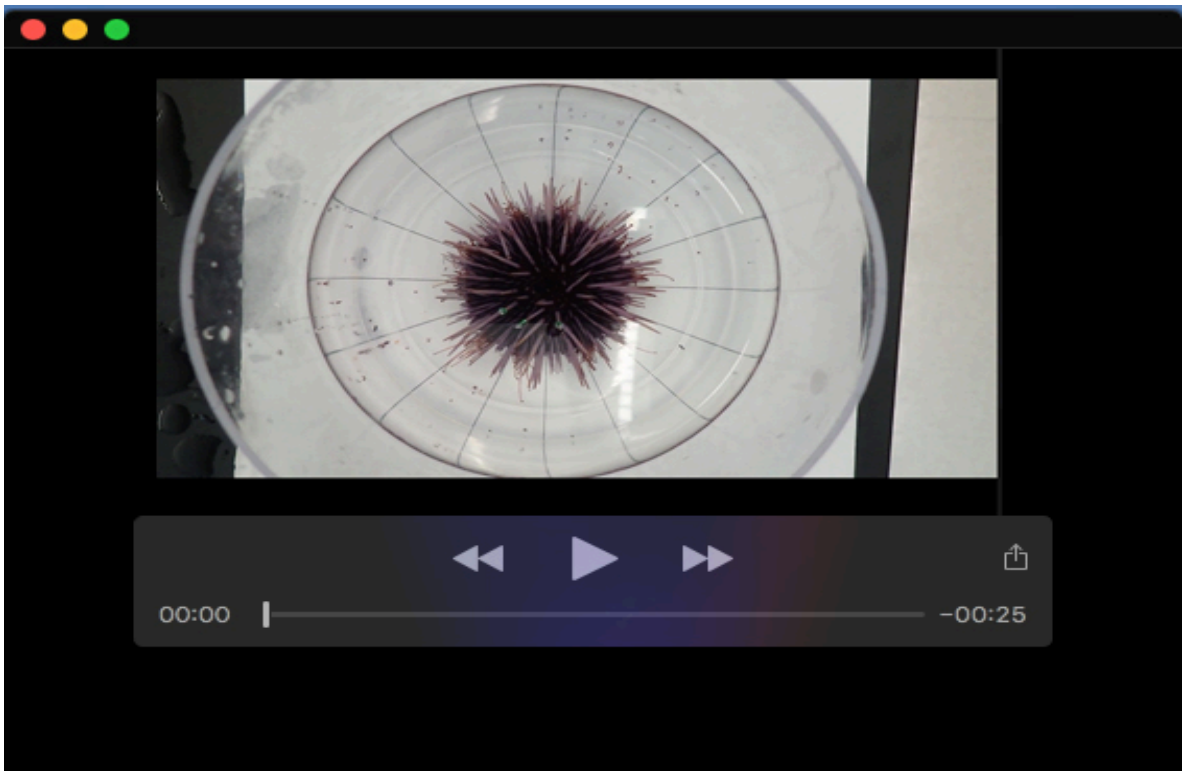

Movie 1. Example tube foot motility assay recording. To assess the motility of tube feet, each sea urchin was immersed in ASW in a circular tank atop of a printed template comprised of a circle divided into 16 equally-sized segments. One-minute recordings were obtained from directly above the sea urchin. Quantification of motility was achieved counting the total number of times any tube foot crossed any line segment (see Methods for details).