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AGONISTIC BEHAVIOUR AND BIOGENIC AMINES IN SHORE CRABS *CARCINUS MAENAS*

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Summary

To investigate the role of certain neurohormones in agonistic behaviour, fights were staged between pairs of size-matched male shore crabs *Carcinus maenas*, and blood samples were taken immediately after the contests had been resolved. Samples were also taken from these crabs at rest (before and after fighting) and after walking on a treadmill. A control group of crabs also had samples taken on each experimental day. Concentrations of tyramine, dopamine, octopamine, serotonin (5-HT) and norepinephrine were determined in each blood sample using a gas chromatography/mass spectrometry (GC-MS) system. Norepinephrine was not detectable in any of the samples, but the standards were recovered. Tyramine values were not significantly different between the control group and the fought group, so tyramine does not appear to be important in agonistic behaviour. A comparison between the control and fought groups shows that fighting had an effect on the concentrations of octopamine, dopamine and 5-HT, but exercise only had an effect on octopamine levels, which showed a reduction from resting values in both winners and losers. Resting and post-fight concentrations of octopamine, dopamine and 5-HT were higher in winners than in losers. 5-HT concentration increased in the blood of fought crabs from resting values, whereas dopamine concentration decreased. In winners, octopamine concentrations decreased from resting values, but in losers octopamine levels increased from resting concentrations. The escalatory behaviour or intensity of fighting performed by winners and losers was related to dopamine levels but not to those of octopamine or 5-HT. Therefore, there appears to be a link between relative concentrations of these three amines (dopamine, octopamine and 5-HT) and fighting ability; the effects are not simply a result of activity. The better competitors have higher concentrations of these three amines at rest and after fighting.

Key words: biogenic amine, aggression, behaviour, dopamine, octopamine, serotonin, shore crab, *Carcinus maenas.*

Introduction

Functional approaches have been successful in elucidating the role of behaviour as a component of fitness, but it is now evident that a full understanding of how natural selection acts on such behavioural traits requires information about the mechanisms that cause them. Game theory models (e.g. Enquist and Leimar, 1983) have incorporated assumptions about the mechanisms that underlie fighting behaviour, and these assumptions need to be tested. These mechanisms may form part of the structure by which fights are resolved and, therefore, it is necessary to link these two approaches by examining physiology in relation to agonistic behaviour in a species (*Carcinus maenas*) in which the fighting behaviour is well studied (Sekkelsten, 1988; Reid et al., 1994; Sneddon et al., 1997a,b, 1998, 1999a,b). Behavioural endocrinologists and biochemists have demonstrated rapid changes in circulating levels of hormones and other behaviourally active substances in response to behavioural experience, including encounters with an aggressive rival (for a review, see Huntingford and Turner, 1987). The present study of shore crabs (*Carcinus maenas*) aims to explore the behavioural roles served by amine neuromodulators, which are believed to alter the duration, intensity and progress of fighting in most clawed decapod crustaceans.

Biogenic amines, such as dopamine, norepinephrine, octopamine, serotonin and tyramine, are important signalling molecules in the nervous systems of all multicellular animals. They are thought to exert hormonal control in a variety of behavioural contexts, including feeding behaviour and aggression (Maler and Ellis, 1987; Kravitz, 1988; Bicker and Menzel, 1989; Coccaro, 1989; Raleigh et al., 1991; Saudou et al., 1994; Weiger, 1997). In crustaceans, biogenic amines function mainly as neurotransmitters and neuromodulators in the nervous system, with some molecules serving as circulating neurohormones as well. Injections of serotonin and octopamine

into the haemolymph of lobsters and crayfish (Livingstone et al., 1980; Yeh et al., 1997) lead to characteristic postures resembling those of dominant and subordinate individuals, respectively. Moreover, infusions of serotonin also affect the level of aggressiveness in lobsters, with subordinates becoming more likely to initiate encounters and less likely to withdraw (Hörner et al., 1997; Huber et al., 1997; Huber and Delago, 1998). Serotonin inhibits the reflex 'escape' reaction mediated *via* the giant lateral interneuron in subordinates and facilitates this 'escape' reaction in dominant and socially isolated animals (Hörner et al., 1997; Krasne et al., 1997; Yeh et al., 1997). Although the behavioural effects resulting from manipulations of aminergic systems are now known to some degree, few studies have attempted to quantify actual *in vivo* circulating levels of these substances in the context of fighting behaviour and locomotory activity in crustaceans.

The objective of the present study is to link amine haemolymph titres to agonistic behaviour in male shore crabs *Carcinus maenas*. To achieve this, concentrations of tyramine, dopamine, octopamine, serotonin (5-HT) and norepinephrine were measured for crabs at rest, after fighting and after walking on a treadmill. One problem with studies that relate levels of biogenic amines to the performance of aggressive acts is that it is hard to distinguish the effects of aggression *per se* from those of locomotor activity. In addition to a non-fought control, the effect of activity on the levels of these amines was assessed, as was the effect of fighting. The present study focuses on inter-individual differences in levels of biogenic amines to explore (1) whether pre-fight concentrations are predictive of fight performance, (2) whether concentrations change as a result of general activity, and (3) whether changes are related to the progress of fighting.

Materials and methods

Animals and experimental protocol

Experimental animals *Carcinus maenas* (L.) (*N*=45; carapace width 55–80 mm) were collected by creeling from the Clyde Sea area and transported immediately to Glasgow, UK, where each crab was placed into an individual holding tank (18 cm×21 cm×23 cm). The crabs were maintained in circulating sea water (34‰) at 10 °C on a 12 h:12 h photoperiod. The animals were measured (carapace width and claw length) on their arrival and kept in isolation for 7 days prior to experimentation since this increases aggressive behaviour. The crabs were not fed during this period to increase motivation to engage in agonistic behaviour. This is a relatively short period of food deprivation since shore crabs can survive for up to 3 months without food (Wallace, 1973). Only animals in good condition were used in this study, i.e. no excessive epibiota, no missing or recently regenerated limbs and no signs of parasitism. Crabs were kept for 2 weeks after the study to ensure that they were not in proecdysis and none was. After this period, crabs were released back into the Clyde Sea.

Crabs were placed into two groups. (1) The control group (*N*=10); these crabs were left undisturbed throughout the experimentation period except to allow the removal of blood samples on days 7, 9, 16 and 18 of captivity. Blood samples (0.5 ml) were taken by piercing the arthrodial membrane at the base of the fourth pereiopod using a syringe and hypodermic needle. (2) The experimental group (*N*=30); these crabs were left undisturbed until day 7, when a blood sample was taken to determine resting values of biogenic amines. The crabs were left for 7 days to ameliorate the variation in energy reserves among individuals (Hill et al., 1991) and to standardize the experience of the crabs prior to sampling, i.e. no social contact with conspecifics because isolation commonly increases aggressiveness (Olivier et al., 1989). On day 9, fights were staged between size-matched (±1 % difference in carapace width and claw length) crabs in an experimental arena (55 cm×28 cm×30 cm) screened from visual disturbance. Each crab was transferred separately to the arena; they were kept apart by a partition which was removed by a pulley system after the crabs had been left to settle for 15 min. Behavioural acts were recorded verbally onto audio tapes using a dictaphone (Sony). These were played back afterwards, and behavioural responses were logged onto an event recorder (acts performed by both crabs). The end of a fight was decided when a definite winner and loser were apparent. The crabs were removed immediately, and a blood sample was taken. The mean fight duration was 352.3±49.5 s (mean ± S.E.M., *N*=15). Shore crab fights consist of a number of discrete acts in which crabs wrestle one another with their abdomen opposed. Acts such as display, in which the crabs stand high on the ambulatory legs and display their claws to an opponent, and contact acts, which comprise potentially injurious acts such as pinching or striking an opponent using the claws, were quantified for the present study. The intensity of a fight was defined by the content of a contest depending upon the behavioural performance of both crabs in a fighting pair. The more potentially injurious acts that were performed by crabs, the higher the intensity (for full details of behavioural acts and intensity of fights, see Sneddon et al., 1997a). The crabs were allowed to recover for 7 days, and another blood sample was then taken from the crabs at rest (day 16). Two days later (day 18), the crabs were placed on a treadmill, and the treadmill was rotated at 2.5 m s^{-1} for 352 s (corresponding to the mean duration of fights). A Perspex box, slightly smaller than the treadmill belt upon which the crab walked, was positioned just above the belt and kept the crab on the treadmill. Thus, the design of the treadmill ensured that the crab walked at this speed. Immediately after the allotted time, a blood sample was taken. All blood samples were immediately placed in an Eppendorf tube and frozen in liquid N_2. The blood samples were stored at −70 °C prior to analysis.

The sequence of experimentation (rest, fight, rest, activity) was chosen to examine the effects of fighting (compared with the first rest sample) and activity (compared with the second rest sample) and also to determine whether the amine concentrations would return to pre-fight levels in the second resting period. Therefore, each individual acted as its own

control. It would have been ideal to have a third group of crabs for which the sequence of experimentation meant that activity was sampled prior to fighting. However, because of limited housing capabilities and finances, this was not possible. The crabs were allowed 2 days to recover from the blood sampling between day 7 and day 9 and also between day 16 and day 18 since shore crabs can make up the blood volume and synthesise the lost blood constituents in 48 h (J. D. Robertson, personal communication). There was 7 day period during which samples were not taken between day 9 and 16 to make these two rest days comparable. The crabs were fed with whitebait after blood sampling on day 9, and they therefore experienced 7 days of isolation and starvation before the next rest sample was obtained on day 16.

Analysis of levels biogenic amines using gas chromatography/mass spectrometry (GC-MS)

The determination of levels of biogenic amines in the blood samples was based upon the methods employed by Watson et al. (1996). The blood sample was derivitised after adding a known concentration of deuterated amines, and the results were compared with those of a standard containing both deuterated and undeuterated amines. The unknown concentration of undeuterated amines can therefore be calculated by comparing the ratios of deuterated amines in the blood sample with the undeuterated and deuterated amines in the standard.

Standard preparation

A standard solution was made by adding deuterated norepinephrine, dopamine, octopamine, 5-HT and tyramine (standard 1) to 1 ml of methanol to give a concentration of 200 ng ml^{-1} of each biogenic amine. Undeuterated and deuterated norepinephrine, dopamine, octopamine, 5-HT and tyramine (standard 2) were added to another vial containing 1 ml of methanol to give a concentration of 200 ng ml^{-1} of each amine. Standard 1 was used as the 'spike' described below. Standards 1 and 2 (0.5 ml) were filtered and derivatised as described below for the blood sample.

Derivitisation and analysis of blood samples

A solution of EDTA (1 mg ml^{-1}) and ascorbic acid (1 mg ml^{-1}) was prepared, 50 µl of this solution was added to the frozen blood sample in the Eppendorf tube, and the sample was allowed to defrost. Blood (0.5 ml) and 25 µl of spike (standard 1 containing only deuterated amines) were placed into an Amicon Centrifree partition device to which 50 µl of 0.3 mol l^{-1} perchloric acid had already been added to make the filter acidic. The device was centrifuged for 5 min (10 000 ***g***). The filtrate was removed by pipette and added to a 3.5 ml sample tube containing 1 ml of phosphate buffer (1 mol l^{-1} potassium phosphate, pH 7.8, in distilled water) and 20 µl of ascorbic acid (10 mg ml^{-1}). To this mixture, 2 µl of 3,5-ditrifluoromethylbenzoylchloride was added, and the mixture was shaken vigorously for 2 min. The aqueous buffer layer was extracted by adding 2 ml of ethyl acetate, and the organic layer was removed to another sample tube. This sample tube was shaken for 30 s after the addition of 0.5 ml of 10 mol l^{-1} ammonium hydroxide. The tube was centrifuged for 1 min (10 000 ***g***), which allowed the two layers to separate. The organic layer was removed and dried by passage through anhydrous sodium sulphate. The ethyl acetate in the resultant liquid was evaporated under a stream of nitrogen gas, and 50 µl of *N*,*O*-bis(trimethylsilyl)acetamide was added to take up the residue. This was left at 60 °C for 15 min, allowed to cool and 50 µl of ethyl acetate added. A portion (2 µl) of this sample was injected into the GC-MS system, and the amounts of tyramine, octopamine, dopamine, 5-HT and norepinephrine were determined by analysing the ratio of undeuterated to deuterated amines in the sample and the standard; values were converted to ng ml^{-1}. Standard addition methods and analysis of pooled samples verified this method. The Sigma Chemical Company (Poole, Dorset, UK) supplied all reagents except the deuterated amines, which were synthesised in the laboratory of D. G. Watson.

Statistical analyses

The results from each biogenic amine were pooled and analysed using a general linear model (GLM; Minitab Version 11). The residuals of data for each amine from the GLM analyses were found to be normally distributed using a normal probability plot, and parametric tests were therefore used to analyse the amine data; *P* values were adjusted for multiple testing throughout (Zar, 1984). The behavioural data were not normally distributed; therefore, non-parametric analyses were applied to the behavioural correlations. A *P* value of less than 0.05 was considered to be statistically significant.

Results

Variations in amine levels among individuals

Norepinephrine was not detectable in any of the samples, although the standards were recovered; norepinephrine was detectable in the standards and from the spike in the blood samples, so failure to detect norepinephrine was not due to the sample derivatisation. Fig. 1 shows mean concentrations of each amine. In the control group, only dopamine concentration (ANOVA, $F_{1,39}$=5.44, P=0.003) was found to be significantly different over the four sampling days, but the data for individuals were significantly concordant (Table 1). Therefore, dopamine concentration was highly variable in each crab, but this variation was similar amongst individuals. Levels of the other amines measured for the control group were found not to be concordant (Table 1). Dopamine was highly concordant in both winners and losers, as was 5-HT, whereas tyramine and octopamine were concordant only for winners and losers, respectively (Table 1).

Experiments took place on four different days, and values obtained from winners and losers were compared with values obtained from the control group (Table 2; Fig. 1). Tyramine concentrations were not significantly different between control crabs and fought crabs (Table 2). Octopamine levels for day 7

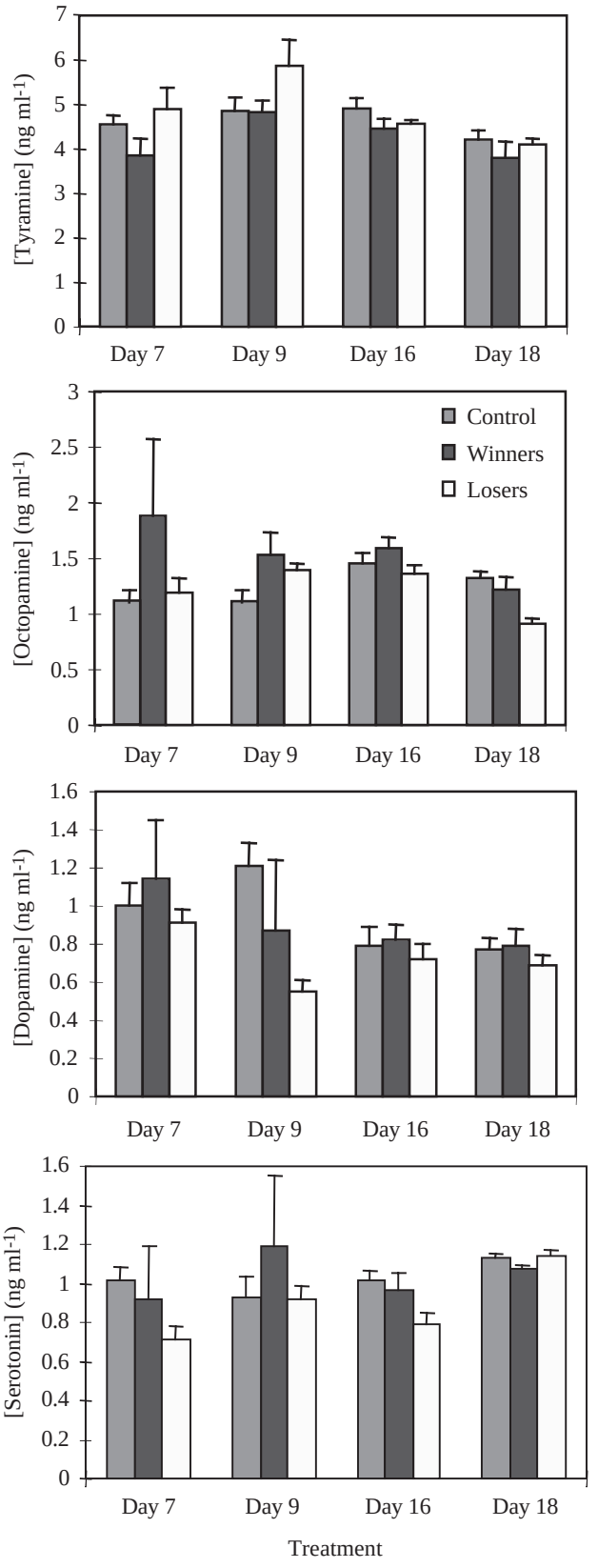

Fig. 1. Concentrations of biogenic amines (ng ml^{-1}) obtained from the control group (N=10) and from winning (N=15) and losing crabs (N=15) on days 7 (rest), 9 (after fighting), 16 (rest) and 18 (after exercise) of sampling. Values are mean + S.E.M.

showed that prospective winners had significantly higher levels than prospective losers ($F_{1,28}$=8.14, P=0.016), for which octopamine levels were similar to those of the control group ($F_{1,23}$=0.47, P=1.00). On day 9, both winners and losers had higher octopamine concentrations than the control crabs (winners, $F_{1,23}$=9.88, P=0.010; losers, $F_{1,23}$=7.75, P=0.020). There was no significant difference in levels of octopamine between winners and losers on day 16 (Table 2), but on day 18 the control group had higher concentrations than the losers ($F_{1,23}$=15.44, P=0.002), which had marginally lower concentrations than the winners ($F_{1,28}$=4.08, P=0.050). Dopamine levels were significantly different between winners and losers on days 7 and 9, when losers had lower levels than winners (day 7, $F_{1,28}$=7.00, P=0.013; day 9, $F_{1,28}$=9.02, P=0.006), but there were no differences between the fought crabs and the control group on day 7 (winners, $F_{1,23}$=1.13, P=0.600; losers, $F_{1,23}$=1.31, P=0.530). There were no differences in dopamine levels between any group on days 16 and 18 (Table 2). 5-HT concentration was higher on day 7 in the control group than in losers ($F_{1,23}$=8.31, P=0.016). However, losers had significantly lower 5-HT levels than winners (day 7, $F_{1,23}$=4.47, P=0.040). On day 9, levels of 5-HT were higher in winners than in losing crabs ($F_{1,28}$=5.98, P=0.021), but there were no significant differences between the control and fought crabs (winners, $F_{1,23}$=3.61, P=0.140; losers, $F_{1,23}$=0.06, P=1.00). On day 16, the control group had higher levels of 5-HT than the losers ($F_{1,23}$=8.91, P=0.016), but concentrations of biogenic amines were similar between winners and losers ($F_{1,28}$=3.75, P=0.140), and on day 18 there were no significant differences in 5-HT levels (Table 2).

Using multiple regression, concentrations of each amine from winning and losing crabs were analysed to determine whether the tested amine levels were different among the four different sampling days and also between winning and losing fights. Tyramine concentration was not related to treatment or fight outcome ($F_{1,77}$=1.62, P=0.21). Dopamine, octopamine and 5-HT levels were significantly related to winning and losing (dopamine, $F_{1,77}$=10.68, P=0.002; octopamine, $F_{1,77}$=7.68, P<0.001; 5-HT $F_{1,77}$=7.76, P=0.001) and also to sampling day (dopamine, $F_{1,77}$=8.75, P<0.001; octopamine, $F_{1,77}$=7.68, P<0.001; 5-HT, $F_{1,77}$=7.76, P=0.001).

To investigate relationships among amine concentrations in the blood of fought crabs, the concentrations obtained after fighting on day 9 were analysed for any correlations using Spearman rank tests. Within losers, tyramine level was positively related to octopamine concentration (Table 3). Between winners and losers, winner 5-HT level was positively related to loser 5-HT level and winner octopamine level was positively related to loser tyramine level and marginally related (P=0.05) to loser octopamine concentration (Table 3). Loser dopamine level was positively related to winner tyramine concentration (Table 3). None of these correlations found in fought crabs existed within the control group on day 9; however, 5-HT and dopamine concentrations were related within the control crabs (Spearman rank correlation, Rs=–0.8018, P=0.005).

Table 1. *Analysis of biogenic amine concentrations from the control, winning and losing crabs showing whether the samples were concordant (P<0.05) on the four sampling days*

		Tyramine		Octopamine		Dopamine		Serotonin	
	N	*W*	*P*	*W*	*P*	*W*	*P*	*W*	*P*
Control	10	0.11	0.36	0.21	0.10	0.53	0.001	0.23	0.08
Winners	15	0.30	0.03	0.08	0.52	0.28	0.04	0.28	0.04
Losers	15	0.22	0.09	0.30	0.03	0.58	<0.001	0.63	<0.001

Results of Kendall's coefficient of concordance (*W*) are shown.

Table 2. *Comparisons of biogenic amine concentrations in the control group and in winning and losing crabs on each day of sampling using one-way analysis of variance*

	Tyramine		OA		DA		5-HT	
	$F_{1,32}$	*P*	$F_{1,32}$	*P*	$F_{1,32}$	*P*	$F_{1,32}$	*P*
Comparison of control group with winners and losers								
Day 7								
C *versus* W	1.99	0.34	4.83	0.07	1.13	0.60	0.81	0.76
C *versus* L	0.20	1.00	0.47	1.00	1.31	0.53	**8.31**	**0.02**
Day 9								
C *versus* W	0	1.00	**9.88**	**0.01**	**7.07**	**0.03**	3.61	0.14
C *versus* L	1.46	0.24	**7.75**	**0.02**	**46.2**	**<0.001**	0.06	1.00
Day 16								
C *versus* W	1.45	0.50	0.97	0.66	0.04	1.00	0.25	1.00
C *versus* L	0.71	0.82	1.21	0.58	0.34	1.00	**8.91**	**0.02**
Day 18								
C *versus* W	0.94	0.68	0.66	1.00	0.03	1.00	3.59	0.14
C *versus* L	0.01	0.93	**15.44**	**0.002**	0.99	0.66	3.19	0.18
Comparison of winners and losers								
Day 7								
W *versus* L	2.55	0.24	**8.14**	**0.02**	**7.00**	**0.01**	**4.47**	**0.04**
Day 9								
W *versus* L	2.19	0.30	**10.87**	**0.01**	**9.02**	**0.01**	**5.98**	**0.02**
Day 16								
W *versus* L	0.66	0.86	4.33	0.10	0.65	0.86	3.75	0.14
Day 18								
W *versus* L	1.18	0.60	**4.08**	**0.05**	1.08	0.62	0.01	1.00
Comparison within winners								
Day								
7 *versus* 9	3.68	0.14	**6.50**	**0.04**	**7.75**	**0.02**	**5.98**	**0.04**
16 *versus* 18	2.19	0.32	**6.36**	**0.04**	0.05	1.00	1.71	0.21
Comparison within losers								
Day								
7 *versus* 9	0.93	0.68	**5.98**	**0.04**	**12.1**	**0.01**	**7.00**	**0.03**
16 *versus* 18	4.08	0.12	**6.47**	**0.04**	0.26	1.00	**19.3**	**0.001**

C, control crabs; L, losing crabs; W, winning crabs; OA, octopamine; DA, dopamine; 5-HT, serotonin.

Comparisons were made between the control group (*N*=10) and winners (*N*=15) and losers (*N*=15) to determine whether winners and losers had significantly different amine concentrations compared with the control group.

To examine the effects of success and failure in fights, comparisons were made between winners and losers on each sampling day.

Comparisons were also made between the first rest day (day 7) and after fighting (day 9) and between the second rest day (day 16) and after exercise (day 18) within winning and losing crabs (values were adjusted for multiple testing).

Any *P* values greater than 1.00 are shown as 1.00 and statistically significant results are shown in bold type.

Table 3. *Correlations among the concentrations of biogenic amines for winners and losers and also between duration and intensity of contests using two-tailed Spearman rank tests*

	Rs	*P*
Duration and W [tyramine]	0.5143	0.050
Duration and L [dopamine]	–0.6750	0.006
Intensity and L [octopamine]	–0.6565	0.008
L [tyramine] and L [octopamine]	0.6071	0.016
W [serotonin] and L [serotonin]	0.5255	0.044
W [octopamine] and L [octopamine]	0.5143	0.050
W [tyramine] and L [dopamine]	–0.6179	0.014
L [tyramine] and W [octopamine]	0.5179	0.048

L, losers; W, winners; *Rs*, Spearman rank correlation coefficient. Only significant results are shown.

Relative concentrations of biogenic amines and performance during fights

There was no significant difference between winners and losers in concentrations of tyramine on day 9 ($F_{1,18}$=2.19, P=0.300; Fig. 1; Table 2). Octopamine, dopamine and 5-HT concentrations were higher in winners than in losers after fighting (octopamine, $F_{1,18}$=10.87, P=0.004; dopamine, $F_{1,18}$=9.02, P=0.012; 5-HT, $F_{1,18}$=5.98, P=0.02).

Lateral merus display and physical contact acts were performed more often by winning crabs than by losers (Friedman tests; winners, S=13.0, P<0.001; losers, S=15.0, P<0.001). The duration of contests ranged from 163.2 to 788.5 s, with a mean value of 352.3±49.5 s (mean ± S.E.M., N=15). Intensity and duration were not correlated (Rs=0.425, P=0.114). Concentrations of tyramine in winners were marginally positively related (P=0.05) to the duration of fights, and dopamine levels in losing crabs were negatively related to the duration of fights (Table 3). Octopamine concentrations in losers were negatively correlated with the intensity of the fight (Table 3).

When the level of escalation was calculated (three different levels based on the crab's behavioural performance during a fight; level 1 is engaging an opponent without injurious acts, level 2 is grasping or pinching an opponent using the claws, and level 3 is striking an opponent using the claws), a stepwise regression showed that only dopamine concentration could account for the level of escalation ($F_{1,24}$=5.08, P=0.032).

A comparison of resting values for the experimental group using ANOVA showed that resting values only differed between days 7 and 16 for dopamine ($F_{1,48}$=8.09, P=0.007). Therefore, octopamine ($F_{1,48}$=0.54, P=0.465), tyramine ($F_{1,48}$=0.22, P=0.638) and 5-HT ($F_{1,48}$=0.60, P= 0.442) concentrations were similar on both resting days.

Effects of fighting and exercise

Mean amine concentrations in both winners and losers are shown in Fig. 1 for each experimental day. Tyramine concentration tended to increase after fighting and tended to decrease after treadmill walking; however, these changes were not significant (Table 2). Octopamine concentration decreased from resting values after fighting in winning crabs ($F_{1,18}$=6.50, P=0.044), but increased in losers (Fig. 1; $F_{1,18}$=5.98, P=0.043). After walking, octopamine level decreased significantly in both winners ($F_{1,18}$=6.36, P=0.042) and losers ($F_{1,18}$=6.47, P=0.040). Dopamine concentration decreased significantly after fighting in winners ($F_{1,18}$=7.75, P=0.022) and losers ($F_{1,18}$=12.12, P=0.010) and tended to decrease after walking, although this was not significant (winners, $F_{1,18}$=0.05, P=1.00; losers, $F_{1,18}$=0.26, P=1.00). 5-HT concentration increased from resting values after fighting (winners, $F_{1,18}$=5.98, P=0.042; losers, $F_{1,18}$=7.00, P=0.028). After walking in losers, there was an increase in 5-HT levels ($F_{1,18}$=19.33, P=0.001), but a similar effect was not seen in winning crabs ($F_{1,18}$=1.71, P=0.21).

Discussion

Circulating levels of biogenic amines in crabs differ significantly within individuals after fighting compared with pre-fight values (i.e. day 7) and also after treadmill walking compared with pre-activity values (i.e. day 16), except in the case of tyramine for which the results are not significant. Norepinephrine was not detectable in these samples, a result similar to that of DeWachter et al. (1997) who did not detect this amine in crabs during normoxia. Larger differences exist between resting and fighting values than between resting and walking values. A comparison of the control group and the experimental group shows that fighting affects the concentrations of octopamine, dopamine and 5-HT, but that exercise only affects the concentrations of octopamine. Levels of dopamine differed between groups on the two resting measurement days, but this is to be expected since circulating levels of amines are highly variable both within and between individuals (S. G. Webster and B. DeWachter, personal communication). Winners had higher concentrations of octopamine, dopamine and 5-HT than losers after fighting. Higher values of 5-HT were anticipated since injection of this amine increases aggression in subordinate crayfish (*Procambarus clarkii*) and elicits the dominant posture in shore crabs (*C. maenas*), lobsters (*Homarus americanus*) and crayfish (*Astacus astacus*) (Livingstone et al., 1980; McPhee and Wilkens, 1989; Huber et al., 1997; Yeh et al., 1997). Increases in octopamine concentration were not expected: injection of octopamine elicits the subordinate posture. However, winners showed decreased levels compared with resting values, whereas losers showed an increase. Octopamine levels of winners were higher than those of losers before fighting (day 7; rest); the same is true for dopamine and 5-HT levels. Therefore, there appears to be a link between levels of these amines and fighting ability, with the better competitors having higher concentrations of these amines. These physiological variables could, therefore, be used as a predictor of fight outcome since, if we know the resting values of these amines, we could potentially predict which crab would be the winner. The escalatory behaviour of winners and losers was

only related to dopamine concentration; this biogenic amine has received little attention with respect to crustacean aggression relative to studies on octopamine and 5-HT and warrants further investigation.

The fights in this study were typical of shore crab contests, with winners performing more display and contact acts and with no demonstrable relationship between the duration and intensity of fights (Sneddon et al., 1997a). Winner tyramine concentration increased with fight duration, suggesting that tyramine is being produced during fights. However, since there are no other statistically significant results with respect to the relative concentrations of this amine and fight outcome, it is unlikely that tyramine plays an important role in fighting behaviour. Concentrations of octopamine in losing crabs decrease with increasing intensity of contests. Octopamine, which has been shown to elicit submissive postures in lobsters *H. americanus* (Huber et al., 1997), may therefore be responsible for de-escalation in losers since it is thought that losers determine fight intensity and possibly duration (Sneddon et al., 1997a). The behaviour of winners and losers during shore crab fights is very different from early on in the contests (Sneddon et al., 1997a); the intensity of contests generally depends upon the strategy the loser adopts. If the loser is submissive during the contests, then the contests are of low intensity. If the loser engages in escalated acts, such as wrestling and striking, then the contests are of high intensity (Sneddon et al., 1997a). The winner appears to respond to the behaviour of the loser and, therefore, if octopamine concentrations are high, the loser behaves submissively or if octopamine concentrations are low the loser performs high-intensity acts and the contest escalates. However, there is no similar pattern in the concentrations of octopamine in winners. Yeh et al. (1997) demonstrated that 5-HT functions differently in the lateral giant neuron of the crayfish *P. clarkii* according to social experience. These amines may also function differently in shore crabs, so similar patterns of amine concentration may not occur in winning and losing crabs.

There are a number of correlations between levels of the different amines in winners and losers after fighting. In losers, octopamine level increases as tyramine concentration increases. Tyramine is the precursor of octopamine and, therefore, it is expected that these concentrations should increase in parallel especially since the losers show an increase in octopamine levels after fighting. As concentrations of 5-HT increase in winners, concentrations also increase in losers, which may be due to fighting for similar lengths of time. However, 5-HT concentration is unrelated to the duration or intensity of contests. Concentrations of octopamine in winners are positively correlated to loser concentrations of tyramine and octopamine, which is to be expected since tyramine and octopamine levels are related in losing crabs. Again, this indicates that the behaviour of winners may be dependent on that of losers. The parallel increases in 5-HT and octopamine concentrations of winning and losing crabs may be because the winner adapts its behaviour relative to that of the loser to establish dominance.

Recent studies have shown that chemical communication may be mediated *via* amine metabolites that are secreted in crustacean urine (Breithaupt et al., 1999). Sulphate metabolites of 5-HT have been identified in lobster *H. americanus* urine (Huber et al., 1997), and concentrations of this metabolite may act as a signalling mechanism indicating dominance status to conspecifics. Winners have higher concentrations of 5-HT than do losers after fighting. Presumably, these concentrations must be restored to pre-fight levels since levels of these dynamic neuromodulators can change rapidly in response to different behavioural situations such as fighting, but it is energetically expensive to create and maintain higher levels of these amines permanently. Therefore, the excess 5-HT following a fight may be excreted in the form of a sulphate metabolite, and excreted levels may be higher for winners to an extent that perhaps the losers can detect. Once a dominance hierarchy has been established in crustaceans, there are few aggressive encounters after initial fighting, with subordinates retreating from the dominant individual (Huber et al., 1997). At the end of a shore crab fight, both contestants disengage, and the loser acts submissively with its claws held in, whilst the winner remains in a raised position and generally does not interact with the loser (L. U. Sneddon, personal observation). Winning crabs also have higher resting levels of 5-HT than do losing crabs; perhaps this amine is secreted in greater concentrations by the winning crab and can be detected by the loser, accounting for the early difference in behavioural strategy shown by losers. Metabolites of octopamine are yet to be found in crustacean urine, but further work is required to identify whether chemical communication is important in the establishment and maintenance of dominance hierarchies. DeWachter et al. (1997) recently suggested that haemolymph L-lactate acted as a metabolic signalling mechanism *via* mediation of levels of biogenic amines that induced behavioural hypothermia in shore crabs. L-Lactate accumulates in the haemolymph and tissues of *C. maenas* after fighting, and physiological mechanisms are therefore important in this species (Sneddon et al., 1998). It would be interesting to investigate whether an interaction between L-lactate and these neurohormones occurs during aggressive interactions.

Fighting has a greater effect on the changes in amine concentrations from pre-fight values compared with the effect exercise has on those from pre-exercise values. This could be because of a reduction in variation in biogenic amine concentrations over the period of this experiment. The present results may provide evidence on the way that these dynamic neuromodulators work. It is thought that biogenic amines are used as short-term up-regulators or down-regulators of behavioural function that influence behaviour, presumably until the neural system has a chance to adapt through changes in gene-expression of receptors or second-messenger responses (Huber and Delago, 1998). This may explain why there is less difference between pre-exercise amine levels (day 16) and exercise levels (day 18) compared with the difference in amine concentrations between pre-fight (day 7) values and after fighting (day 9) and why the variation (S.E.M.) in dopamine,

octopamine and 5-HT levels in the control group decreased over the experimental period. A recent study on lobster (*H. americanus*) aminergic neurons responsible for posture has shown that increased levels of 5-HT and octopamine significantly changed the firing pattern of neurosecretory neurons over periods of minutes, so biogenic amines actually play a role in the regulation of the neural circuitry (Heinrich et al., 1999).

Biogenic amines have been shown to serve various physiological and behavioural roles (for a review, see Fingerman et al., 1994). Therefore, these marked increases or decreases in amine concentration after fighting should have profound effects upon the animal. 5-HT, for example, has been shown to be involved in pigment dispersion (Bauchau and Mengeot, 1966), in stimulating moult-inhibiting hormone production, in triggering gonad-stimulating hormone, in increasing heart rate by increasing cyclic AMP levels in heart muscle and exciting the cardiac ganglion (Hoeger and Florey, 1989), in modulating the neural circuitry that generates the pyloric rhythm, in influencing the postural system by causing leg flexion, in increasing ventilation rate, in reducing photonegative behaviour and in elevating glucose levels in the haemolymph. The effects that may be important in fighting behaviour are postural control, where display is an important part of aggressive interactions, increases in heart and ventilation rates, as shown in velvet swimming crabs *Necora puber* after fighting (Smith and Taylor 1993), and elevated glucose levels, which have been demonstrated in *C. maenas* after fighting (Sneddon et al., 1998, 1999a). This is a complex subject that requires more attention; far too little is known of the specific effects of these neurohormones, which probably do not have single sites or targets of action in the nervous system concerned with aggressive behaviour. Another aspect that should be addressed is the effects of prior experience on an agonistic encounter and on amine concentrations. Experiments involving pharmacological blocking or enhancement of the production of these amines are needed to prove that these amines are directly responsible for the observed differences in behaviour and competitive ability of individuals. It is thought that neuromodulators may coordinate the activity of neural decision-making centres and hence influence the motivation of an animal (Nader et al., 1997). This is an exciting area of research that may bridge the gap between causation and function in behavioural studies and perhaps clarify underlying physiological mechanisms responsible for aggressive behaviour.

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