INTERNEURONES IN CRAB CONNECTIVES (CARCINUS MAENAS (L.)): DIRECTIONAL STATOCYST FIBRES

BY PETER J. FRASER

Department of Neurobiology, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra City, A.C.T. 2600, Australia

(Received 24 April 1974)

SUMMARY

Five interneurones in each connective respond directionally to rotation of a crab. Each seems to be activated by one direction of fluid flow in one statocyst canal. The anatomy of two is known and correlates well with a direct input from the receptors of one statocyst. Three of the fibres have input from leg joint proprioceptors.

The swimming reflex and a complicated turning reflex are evoked on rotation of a minimally restrained crab. It is likely that the statocyst interneurones are involved in these and other behaviour patterns, including the rearing reflex and sideways walking.

INTRODUCTION

Stereotyped behaviour patterns evoked by linear acceleration (including gravity) or angular acceleration acting on the statocyst are well known. Compensatory eye and appendage movements to tilt round the horizontal and vertical axes have been studied in a variety of decapods (Schone, 1954, 1961, 1971; Cohen & Dijkgraaf, 1961; Davis, 1971; Sandeman & Okajima, 1972, 1973a, b). Furthermore, the effects on behaviour following unilateral or bilateral statocyst ablation in the crab suggest statocyst involvement in many other behaviours, including sideways walking (Bethe, 1897a, b). Crabs are known to have a complicated statocyst with vertical and horizontal canals for rotation reception as well as a statolith for gravity reception (Sandeman & Okajima, 1972).

Studies on statocyst-driven behaviour, like other studies on crab behaviour, have little knowledge of interneurones to support them. Roye (1972) describes large cells with axons in the oesophageal connective of Callinectes responding to directional movements of the antennule base. In the crayfish, Wiersma & Mill (1965) describe three phasic units with input from the statocyst. In neither case have these units been studied during rotation of the animal round the vertical and horizontal axes, even though one of the crayfish units has been studied in free-walking animals (Taylor, 1970).

This paper describes five interneurones in each connective of Carcinus which respond directionally to rotation of the animal round the various axes, and certain behaviours evoked by the same rotations.
Fig. 1. The crab is clamped between perspex jaws in a framework suspended by three strings. Two micromanipulators clamped to the framework hold suction electrodes or as shown, a microelectrode (right) and a glass hook (left) to steady one connective.

MATERIALS AND METHODS

Animals were obtained, kept and prepared for electrical recording as described in the preceding paper (Fraser, 1974). The crab was clamped in a frame (Fig. 1) which was suspended in air by means of three strings. Two micromanipulators were attached to the frame, allowing electrical recordings with suction electrodes or microelectrodes to be made. Stable microelectrode recordings could be obtained while the crab was rotated round the rolling, pitching and yawing axes.

Pitching (rotation round the horizontal transverse axis) was performed by raising or lowering the front string by hand. Yawing was achieved by rotating the apparatus slightly round the vertical axis and letting it oscillate from this displaced position. Faster forced oscillations were obtained by hand. Rolling (rotation round the horizontal longitudinal axis) was performed by lifting the whole apparatus clear of its supporting strings and turning it by hand.
Interneurones in crab directional statocyst fibres

Fig. 2. Suction electrode record from the brain side of the right connective showing the five statocyst fibres. All recordings go from left to right.

(a) Rotation round the horizontal transverse axis. The large unit (A) fires on head-up rotation (lower line up). Two smaller units (C, D) fire on head-down rotation (lower line down).

(b) Rotation round the vertical axis. The larger unit (B) fires on anticlockwise rotation (lower line up). The small unit (E) fires on clockwise rotation (lower line down).

(c) Rotation round the horizontal longitudinal axis. Unit C fires on right-side-down rotation (lower line up) and D fires on left-side-down rotation (lower line down). Unit A which fires on right-side-down rotation round this axis has habituated.

Although the animal was suspended in air, with only a saline-drip providing moisture, stable recordings could be made for several hours. Furthermore, the statocyst units were unaffected by cutting the cerebral artery (which quickly causes visual responses to disappear). The artery was hence often cut to eliminate interfering visual units from the records. Anatomical results were obtained by dye injection as in Fraser (1974).

RESULTS

Five units and their statocyst input

Extracellular suction electrode records from the brain side of both connectives show five units in each connective which fire on rotation of the animal (Fig. 2). In typical order of amplitude of extracellular spike, A being the largest, they are as follows:

(A) Responds on head-up rotation round the horizontal transverse axis and same-side-down rotation round the horizontal longitudinal axis. A is the giant fibre of 5 of Fraser (1974), as will be seen later.

(B) Responds on clockwise rotation round the vertical axis in the left connective, pd anticlockwise rotation in the right connective.
Table 1

<table>
<thead>
<tr>
<th>Unit</th>
<th>Connective</th>
<th>Axis and direction round which unit responds</th>
<th>Statocyst providing main input</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Horizontal transverse</td>
<td>Horizontal longitudinal</td>
</tr>
<tr>
<td>A</td>
<td>Left</td>
<td>Head up</td>
<td>Left side down</td>
</tr>
<tr>
<td>A</td>
<td>Right</td>
<td>Head up</td>
<td>Right side down</td>
</tr>
<tr>
<td>B</td>
<td>Left</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>Right</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>Left</td>
<td>Head down</td>
<td>Left side down</td>
</tr>
<tr>
<td>C</td>
<td>Right</td>
<td>Head down</td>
<td>Right side down</td>
</tr>
<tr>
<td>D</td>
<td>Left</td>
<td>Head down</td>
<td>Right side down</td>
</tr>
<tr>
<td>D</td>
<td>Right</td>
<td>Head down</td>
<td>Left side down</td>
</tr>
<tr>
<td>E</td>
<td>Left</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>Right</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

(C) Responds on head-down rotation round the horizontal transverse axis and same-side-down rotation round the horizontal longitudinal axis.

(D) Responds on head-down rotation and opposite-side-down rotation round the horizontal axes.

(E) Responds to anticlockwise rotation round the vertical axis in the left connective and clockwise rotation round the vertical axis in the right connective.

The contribution of the statocysts to these units can be seen by unilateral or bilateral statocyst ablation (either cutting the antennulary nerves, or removing the whole antennule). Removal of the anterior part of either or both antennules has no effect on the units. Removal of the left statocyst, for example, causes units A and D in the right connective, and units B, C and E in the left connective to stop firing to their appropriate rotations. Removal of both statocysts causes all the units to stop firing. The units thus each have input from one statocyst. The responses are summarized in Table 1.

In each connective the five units identified as above can be recognized individually by the appropriate rotation, and hence are easily identified in single unit records with the nervous system intact.

Additional inputs

(a) Leg joints

With the nervous system intact, cells A, C and D fire on forced or voluntary movements of the walking legs as well as to rotation. Cells B and E have no input from the legs. A responds to movements of any of the walking legs upwards (principally around the coxopodite-basi-ischiopodite (C-B) joint, but also about the thoracic-coxopodite (T-C) joint). The response adapts quickly and is very variable for a given joint. It is better ipsilateral. Summing inputs from opposite sides, by raising one leg on one side together with any leg on the other side, usually gives a large response. The cell fires very reliably to such summation of both sides (Fig. 3). Cutting one connective eliminates this summed response. This, together with the fact that the unit is descending (Fraser, 1974), suggests that sensory information from the joints travels up on either side to the brain where it sums to fire the cell. C responds to upwards movement of any of the legs at the C-B and T-C joints, but does not sum the information from the two sides non-linearly as fibre A does. D responds to down
wards movement of any of the legs about the C-B joint, without any non-linear summation of input from both sides. Cells A, C and D also give a maintained tonic discharge during 'spontaneous' struggling movements of the legs.

(b) *Spontaneous*

With the animal in sea water there is usually a sporadic discharge from cell A with occasional bursts of spikes on antennule withdrawal. With the crab out of water (as was usual when recording during rotation) there tended to be less spontaneous activity. Some preparations still had a spontaneous level of discharge. This was cut off abruptly upon head-down rotation. Cells C and D often were spontaneously active as well, giving regular tonic discharges which were only inhibited on head-up rotation.

(c) *Antennule movement and vibration*

All five cells fire on antennule withdrawal, and as this is the most readily evoked of the head appendage reflexes (Bethe, 1897a, b), spikes to stimuli such as light off and on (which cause withdrawal) were common from all five cells, although no direct visual input was ever shown. All five fibres are also sensitive to vibration.
Fig. 4. Number of spikes from fibre A in the right connective (clear) and left connective (black) to oscillations (rotation round the horizontal transverse axis) damped only by air resistance. The unit fires only on the swing forward (i.e. head-up rotation). Numbers of spikes in both connectives fluctuate considerably. Changes in illumination had no effect on output.

**Habituation**

All five units habituated to a repeated rotation. This habituation was often variable. Cell A habituated most rapidly, the response to the same side-down rotation disappearing before that to head-up rotation. Often the response would habituate completely after only three oscillations. At other times the cell would show little habituation at all. In a well-rested animal there was a long after-discharge on halting head-up rotation which was terminated on head-down acceleration. The other four fibres showed similar habituation, the smallest unit habituating slowest.

The excitability of the fibres varied considerably. In one experiment to investigate such variability, a response from cell A was recorded extracellularly from each cut connective, and the animal in its frame was allowed to oscillate backwards and forwards (i.e. producing rotation about the horizontal transverse axis). The period of oscillation was about 2 sec. If the response to a given rotation was constant, then the number of spikes per oscillation should have gradually declined (as friction lowered the amplitude of oscillation). In fact, spike output in left and right cells varied extensively and independently (Fig. 4). This variation was independent of ambient light intensity.
Anatomy

Only the anatomy of cells A and C is well established. The suspected anatomy of cells B, D and E is given elsewhere (Fraser, 1973). The location of all the axons in the connective is known reliably and is shown in Fig. 5. It can be seen that cell A is giant fibres 5 (see fig. 15, plate 1, Fraser, 1974).

Cell A

The whole mount horizontal plan is shown in Fig. 6. The main part of the cell continues into the brain from the connective, passing lateral to the tract of fibres from the ventral posterior cells. It turns at right angles and crosses over to the other side of the brain in the tract of fibres containing cell 6 (Fraser, 1974) just posterior to the cerebral artery. On the other side of the brain, the main dendrite-carrying portion runs anteriorly and posteriorly at 45° to the longitudinal axis. The posterior branches are ventral, with processes in the lateral antennulary neuropile, the tegumentary neuropile and the antennary neuropile. The anterior branches remain in the thick-fibred dorsal part of the brain, clear of the discrete thin-fibred neuropiles. The neurite to the cell body runs dorsally to the dorsal anterior medial cells giving off several processes close beside processes from the main anterior branch.

Cell C

This cell has its cell body on the ventral side of the dorsal anterior medial cell group on the same side as its axon (Fig. 7). The main dendrite-carrying part runs at 45° to the longitudinal axis forming a near mirror-image to that of cell A. Branches are given off anteriorly to the thick-fibred portion of the brain above the optic neuropiles (with one branch descending into the optic neuropiles) and posteriorly to the lateral oculomotor and lateral antennulary neuropiles.

Free-walking animals

In free-walking animals with implanted electrodes (see Fraser, 1974) the fibres could all be identified by picking the animal up and rotating it. The response patterns were all similar to those obtained in the restrained preparation. Rhythmic discharges from fibre 5 were obvious during sideways walking, and activity was also present during the rearing-up part of the rearing reflex.
Fig. 6. Horizontal plan (camera lucida drawing) of unit A in the brain.

Fig. 7. Horizontal plan (camera lucida drawing) of unit C in the brain.

Fig. 8. The position of threads a, b and c glued to the carapace and the abbreviations of appendages used in the text.
Behaviour

One method of correlating unit activity with behaviour is to observe what kinds of behaviour are elicited by sensory stimulation adequate to fire the unit, so it is appropriate to look at reflexes evoked by rotation of crabs round the various axes. Rotations of the animal were achieved by means of threads glued to the carapace (Fig. 8), since undue contact of the animal with apparatus could inhibit reflexes. Various observations have been made on crabs suspended by threads: Carcinus performs erratic leg movements and Portunus (= Macroopus) performs organized swimming movements on loss of contact with the substratum (Lochhead, 1961). Carcinus will also swim (usually with not enough lift to counteract gravity) when dropped into deep water.

Whole animal

Pitching the crab head down produced immediate raising up of both legs $L_8$ and $R_8$ (abbreviations for legs are shown in Fig. 8) over the dorsal carapace. The legs were extended about the protopodite–dactylopodite (P–D) and the meropodite–carpopodite (M–C) joints as they were raised, and then rotated in a cyclic fashion 180° out of phase as for swimming. Meanwhile the other legs were raised slightly about their basal joints and the claws brought forwards and inwards, completing the full swimming posture as described by Hartnoll (1971) and Bethe (1897a, b). Often the swimming involved paddling movements of legs 2 and 4 in addition to last-leg rotation. This swimming reaction could be very reliably evoked by any head-down rotation and continued until the crab was rotated head up, back to its original position. Head-up rotation inhibited the swimming. Slight head-up rotation stopped swimming in a crab held with its head down, but swimming resumed in the new position which was still head down relative to normal. On repeated oscillation between head up and head down, the response eventually habituated. The upwards kick of the back legs was the last component to habituate.

On rolling the animal the second, third and fourth legs on the raised side were extended and held out. The fifth leg on this side beat rhythmically as for swimming, and legs 2, 3 and 4 on the lowered side were flexed and extended cyclically. Legs 2 and 4 tended to move together with leg 3 in opposite phase. No effects on the legs were visible on yawing the animal.

These reflexes were unaltered by covering the eyes with black Plasticine, and were easily evoked in air and water. The pitch reflex was stronger and more easily performed than the roll reflex. Small crabs performed both reflexes more readily than large crabs.

Antennule removal

The anterior parts of both antennules could be removed with no effect on either of these reflexes. On removal of the left basal part containing the statocyst, various effects were noted. On head-down rotation $R_8$ was now more mobile than $L_8$, and, although the swimming reaction happened apparently normally in a rested animal, only the $R_8$ kick was evoked as the reflex became adapted. During swimming, as well as rotating the last legs, the crab tended to beat the remaining legs on the left. The animal always tended to turn anti-clockwise. On raising the animals left side, it at
first gave no response, but then gave a peculiar cyclic beating of the left legs. Raising the animals' right side produced no motion. The animal seemed to 'freeze'.

After removal of both antennules further effects were apparent. There tended to be a lot of spontaneous swimming. On head-down rotation the normal swimming reflex was still present but habituated far more quickly than in the intact animal. On raising one side the legs of the lower side flexed and extended cyclically. Those of the upper side did not extend. Covering over both eyes reduced but did not abolish these responses. They could also still happen in both air and water. Input from leg receptors seemed then to be involved. The continued response in air seemed to suggest that joint receptors rather than hairs on the leg were involved. After several weeks of being suspended by threads in sea water, the reaction to rotation became progressively more and more reduced. When a suspended crab was eventually released after 4 weeks, its posture was peculiar: it tended to keep its head very much lower than a normal animal.

All behaviour patterns are dependent to some extent on the orientation of the animal, so it is appropriate to look for statocyst mediated effects not only in equilibrium reflexes, but over a wide range of behaviour patterns. This can be done most easily by unilateral or bilateral statocyst ablation and has already been done in *Carcinus* by Bethe (1897a, b). All Bethe's results were readily confirmed here. Attention is drawn to one reflex—the rearing reflex (Aufbaureflex). Animals with both or one statocyst intact perform a normal rearing reflex. Animals with both statocysts removed perform a normal reflex with the exception of the back legs. These are not held in the normal flat stable position, but are held like the other legs on their tips, and not so far back as usual. The result is that, as the animal rears to track an approaching object, it falls over backwards. In the normal animal also, if the crab performing the rearing reflex is pushed on the anterior carapace in such a way that the force of the push tends to turn the crab head up round the horizontal transverse axis, its back legs react by being pushed backwards to counteract this rotation. In the animal with no statocysts under the same circumstances there is still a last leg movement on pushing the animal, but it is not directed enough to stabilize the animal, so that the crab falls on its back.

**DISCUSSION**

The statocyst interneurones described here have more easily described sensory fields than the interneurones described in the previous paper (Fraser, 1974) and can be implicated in the various postural and locomotory behaviour patterns controlled and driven by the statocysts. These behaviour patterns are basic to all other forms of behaviour and their stereotyped nature and the ease with which they are evoked simplify analysis. The interneurones described here have many properties necessary for subunits of postural and locomotory behaviour, being capable of monitoring rapid changes in leg and body position, both for correcting errors and providing phase information when rotational stimuli are generated naturally during a behaviour pattern.

The working of the statocyst is not fully understood (for instance, no function of free hook hairs is known and dynamic responses from the statolith hairs are not fully investigated). Moreover a complication is that linear acceleration responses from
Angular acceleration receptors have been recently discovered (see Fraser & Sandeman, in preparation). However, the responses of the interneurones described here can be explained on a basis whereby angular acceleration is detected by fluid movement, relative to the horizontal canal for rotation round the vertical axis, and relative to the vertical canal for rotation round the horizontal axes. The separation of units detecting rotations round vertical and horizontal axes supports this explanation, furthermore the ambiguous responses of cells A, C and D to rotation round the horizontal axes cannot easily be explained in any other way. Sandeman & Okajima (1972) predicted that the morphology and orientation of the statocysts were such that fluid flow in the vertical canal occurs during rotation round both horizontal axes. Thus head-up rotation and right-side-down rotation should produce identical fluid flows (and hence receptor stimulation) in the left statocyst. Cell A in the right connective, which is supplied by the left statocyst, responds to head-up rotation and right-side-down rotation, and therefore must be excited during fluid flow in the left statocyst vertical canal. In a similar way cells C and D in both connectives, and cell A in the left connective, are each excited during one direction of fluid flow in the appropriate statocyst. Where one statocyst confuses two directions of rotation the other discriminates between them (Sandeman & Okajima, 1972). The confused information is sent down each connective, so both connectives are necessary to transmit complete rotational information to the thorax via these large fibres. Given that both connectives must be symmetrical for growth reasons, this is perhaps the way the animal exploits the bilateral symmetry in the nervous system.

The directionality of the interneurones needs to be explained. The statocyst contains three classes of receptor (Sandeman & Okajima, 1972), thread hairs, free hook hairs and statolith hairs, and of these the thread hairs are the receptors which we infer excite the interneurones. These thread hairs are said to be responsible for the dynamic component of various statocyst driven behaviours (Cohen & Dijkgraaf, 1961; Schone, 1961; Sandeman & Okajima, 1972, 1973 a) and have been proved to drive the eyes during rotation of the animal. Blinded animals with all except the thread hair nerves to both statocysts cut still perform normal compensatory eye movements; furthermore, when the thread hair nerves on both sides are cut, with all other statocyst nerves intact, compensation is far slower than normal (Cohen & Dijkgraaf, 1961). Sandeman & Okajima (1972) found that unit discharges from thread hairs were directional. The most likely explanation is that each unit receives input only from thread hairs orientated in an appropriate direction, but excitatory or inhibitory interactions involving the different outputs from the two main groups of thread hairs or from hairs with different preferred direction (Sandeman & Okajima, 1972) are likely to occur.

Phasic statocyst interneurones in the crayfish are known (Wiersma & Mill, 1965). The responses of these fibres during rotation of the animal are not known, and direct input to these fibres from leg proprioceptors has not been indicated. In the oculomotor system motoneurones in the crayfish or rock lobster are not stimulated on angular acceleration, whereas motoneurones in the crab are (Wiersma & Fiore, 1971 a, b). The crayfish has a spherical statocyst simpler than that of the crab (Schone, 1971) and the lack of separate canals may well correlate with a lack of directional rotational information. A fluid-filled sphere containing directionally polarized hair
receptors is theoretically as capable as a canal system of providing directional rotational information, but such a system may be more susceptible to noise in the form of convection or eddy currents than a canal system, thus losing the directional information.

The input from the leg joints on to cells A, C and D modifies the rotational signal. In the case of cell A, the inputs from legs on either side sum non-linearly. This summation does not happen when either connective is cut, showing that the leg proprioceptive signals on either side go up the ipsilateral connective before interacting in the brain. Statocyst, and right and left leg inputs must also sum (perhaps non-linearly), making the cell extremely sensitive to a combination of head-up rotation or same-side-down rotation with upward bending of the basal segments of right and left legs. Cells C and D will be similarly sensitive to their respective combined inputs, of head-down rotation or same-side-down rotation with upwards bending of the legs, and head-down rotation or opposite-side-down rotation with downwards movement of the legs. These combined inputs occur during sideways walking.

The anatomy of cells A and C is established and supports physiological evidence for an input arrangement closely associated with the receptors of one statocyst. Most of the dendrites and thickened integrating segment of cell A (which is excited by the ipsilateral statocyst) is ipsilateral. Sensory nerves from the statocyst all terminate ipsilaterally (Sandeman & Okajima, 1973), near the dendrites of the interneurones. The possible input locations of leg proprioceptive information and the extent of other input from the statocyst via interneurones are unknown.

The interneurones described here are adequate to carry the rotational information from the statocyst down to the motor centres in the thorax, but other statocyst interneurones not so far discovered are undoubtedly present. The large fibres have features such that they could activate and control dynamic aspects of postural and locomotory reflexes, but smaller fibres with input from the statolith are required to control static aspects of the reflexes. In free-walking animals (Fraser, 1974) the fibres are active during the reflexes described below.

During the rearing reflex (Aufbaumreflex) an animal without statocysts does not direct its last legs well enough to compensate for the head-up rotation involved in tracking an approaching object, and it often falls over. Upwards movement of leg joints and head-up rotation are combined during this tracking, providing strong input to cell A. It is proposed that cell A is responsible for this stabilizing control of the back legs. A single statocyst (and hence a single cell A) is adequate to control both last legs in this behaviour pattern.

The role of the statocyst in swimming has been demonstrated by suspending the animal on several threads. Head-down acceleration evokes swimming. This reaction depends on information from the legs and eyes as well as the statocyst (a similar control of compensatory eye movements has been indicated by Schone, 1961). The existence of this swimming reflex as a true equilibrium reflex stabilizing a crab falling through the water cannot be doubted. The importance in an environment such as that inhabited by Carcinus is obvious. Carcinus following an advancing tide up a beach has to compensate for wave action lifting and turning it. We now see that the specializations of very mobile flattened back legs and lightened carapace for swimming in the Portunidae can aid this reflex, which is performed by all crabs (Hartnoll, 1971). Cells C and D are excited by the stimuli causing swimming and form a likely pathway...
For the initial activation of the swimming reflex, stimulation of the part of the connective containing the axon of cell C causes the full swimming reflex (Fraser, 1973). Swimming is also under inhibitory control from the brain, as removal of both statocysts causes an increase in the amount of spontaneous swimming.

The equilibrium reactions evoked on rotation about the horizontal longitudinal axis are very complicated, although films may reveal that they are part of co-ordinated turning. The fifth leg on one side is apparently controlled along with legs 1–4 on the other side. Thus the lowered side legs 1–4 beat cyclically and the raised side leg 5 also beats. In the swimming crab *Portunus* (*= Macropipus*) on cutting the connective, legs on the homolateral side have an increased flexor tone, those on the heterolateral side an increased extensor tone, but exactly the opposite reactions appear in the two swimming legs (Wiersma, 1961).

Although crab locomotion is currently under intensive study (Evoy & Cohen, 1971; Barnes, 1973) many of the old results concerning the role of the statocysts and the brain in sideways walking (Bethe, 1897b; Wiersma, 1961; Cohen & Dijkgraaf, 1961; Schone, 1961; Lochhead, 1961) are not incorporated into modern work. Sideways walking in *Carcinus* cannot occur without the brain, or more specifically without the dorsal anterior medial cells (Bethe, reported in Schone, 1961). Cells A and C have their cell bodies in this group. The combined inputs capable of exciting cells A, C and D occur during walking. It is proposed that these cells provide dynamic integrated information on leg and body position during walking. Cells B and E have properties such that they could correct steering errors.

I am grateful to Dr D. M. Guthrie for his supervision and encouragement, to Jonathan Banks for technical help, to Jean Smith for translating Bethe's work and to Prof. G. A. Horridge and Dr D. C. Sandeman for their criticism of the manuscript. The work was done at Aberdeen University, Zoology Department, and supported by a grant from the Science Research Council.

**REFERENCES**


