Chemical Senses Vol. 14 no. 3 pp. 439–448, 1989

Adaptation of male moth antennal neurons in a pheromone plume is associated with cessation of pheromone-mediated flight

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Abstract. Recordings of the firing rates of single antennal neurons when *Agrotis segetum* antennae were placed 70 cm downwind of a pheromone source revealed that cells sensitive to the most volatile component adapted rapidly in a plume from a high-concentration source known from previous studies to cause in-flight arrestment of progress towards the source. No adaptation was found in response to lower-concentration plumes known to promote high levels of sustained flight to the source with little premature arrestment. Adaptation was not observed in antennal neurons of a second species, *Heliothis virescens*, when they were placed in plumes of this species' sex pheromone blend, regardless of the concentration. In flight-tunnel tests these same pheromone sources evoked high levels of source location with little arrestment. These results indicate that adaptation or attenuation of antennal neuronal burst frequencies in response to rapidly arriving pheromone filaments in a plume may be important peripheral determinants of whether or not prolonged upwind flight and successful pheromone source location occurs.

Introduction

As male moths fly in response to a source of their sex pheromone, they may repeatedly switch from making upwind progress to ceasing this progress, then back again, all in a matter of fractions of seconds (Baker and Haynes, 1987). These periods of momentary arrestment may be caused by drops in concentration when the wind shifts direction and the moth flies into clean air. The moth pauses in mid-air when the odor is lost rather than progress upwind, because to continue such progress would take the moth in a direction other than toward the source (David *et al.*, 1983). The station-keeping and wide tracks that follow shortly after, during what is called casting flight (Kennedy, 1983), allow the male eventually to relocate the plume and continue upwind. Arrestment of upwind progress also is known to occur in plumes from point sources that emit excessive amounts of the correct blend of components or incorrect ratios of components at proper emission rates (Willis and Baker, 1988). Suboptimal blends and excessive emission rates may be emitted by non-conspecific females and encountered in natural settings by male moths, and arrestment of upwind progress would prevent males from making interspecific mating mistakes.

The requirement for intermittent pheromone stimulation in sustaining upwind flight has only recently been demonstrated behaviorally in some species (Kennedy *et al.*, 1981; Baker *et al.*, 1985), even though researchers have long been aware of the broken, filamentous fine structure of airborne pheromone plumes that might contribute to such intermittency (Wright, 1958; Murlis and Jones, 1981). The upwind progress of males of some species becomes arrested in flight in uniform clouds of pheromone (Kennedy *et al.*, 1981; Baker *et al.*, 1985), and adaptation or habituation have been suspected as the cause (Kennedy *et al.*, 1981). It has also long been speculated that as male

moths fly upwind in a pheromone plume to locate a female, sensory adaptation of their receptors or habituation of sensory pathways may be significant factors in their success in locating a female (Shorey, 1970; Bartell, 1977). There has, however, been no direct neurophysiological evidence indicating that either adaptation or habituation can occur and interfere with flight in a pheromone plume, because few cellular recordings have been performed which utilize the kind of rapidly fluctuating exposure to pheromone that occur when the moth is in a wind-borne plume (Rumbo, 1983; Kaissling, 1986), and no studies have correlated neuronal output from such stimulation with upwind flight. Therefore, we recently recorded from the single antennal neurons of two moth species and challenged the neurons with rapid pulses of pheromone by placing the single-cell preparation directly into the pheromone plume itself and recording for the first time the responses of individual neurons as they might fire during flight in a plume.

Materials and methods

All neuronal recordings were performed extracellularly using the cut-sensillum technique of Kaissling (Kaissling, 1974; Van der Pers and Den Otter, 1978). An antenna was excised from the head, and the antennal base placed in a saline-filled pipet ground electrode. The tip of a sensillum trichodeum was cut with a glass knife and then contacted with a saline-filled pipet recording electrode. Neuronal responses were recorded in both AC and DC. Male antennae from two species were used, *Agrotis segetum* (Schiff.) and *Heliothis virescens* (F.). For all neuronal responses, a behaviorally active complete blend of the pheromone components of each species was always used. For *A. segetum* the blend was a 0.6:1:5:2.5 ratio of decenyl acetate (*Z*)-5-decenyl acetate (Z5-10:Ac), (*Z*)-7-dodecenyl acetate (Z7-12:Ac) and (*Z*)-9-tetradecenyl acetate respectively (Löfstedt *et al.*, 1985). Serial dilutions were made in hexane such that 0.3, 3, 30 and 300 μg of Z5-10:Ac were applied to rubber septum dispensers. The first three dosages were all known to promote sustained upwind flight to the source, whereas the 300 μg loading had been shown to be excessive, causing cessation of upwind flight in nearly 100% of the males that had begun upwind flight farther downwind in a flight tunnel (Löfstedt *et al.*, 1985). For *H. virescens*, the blend was a ratio of 100:18:8:10:1:1 of (*Z*)-11-hexadecenal (Z11-16:Ald), hexadecanal, (*Z*)-9-tetradecenal, tetradecenal, (*Z*)-9- and (*Z*)-7-hexadecenal respectively (Pope *et al.*, 1982). Serial dilutions were made in hexane, and 10, 100, 500 and 1000 μg of (*Z*)-11-hexadecenal were applied to rubber septa dispensers.

Recordings of single antennal neurons while in a pheromone plume were performed by placing the antennal preparation 70 cm downwind of the pheromone source at the end of a small wind tunnel, 80 cm long × 20 cm i.d. Wind was generated at 0.5 m/s in the tunnel at 22°C. When the sensillar preparation was ready, the specificity of the neurons in that sensillum for a particular component was determined by applying puffs from cartridges containing 1 μg of a single component. The preparation was then positioned 10 cm from the end of the tunnel. A rubber septum containing the lowest dosage of the pheromone blend was then placed on a wire and inserted into a small hole 70 cm upwind of the antenna. A point source of smoke had indicated that the plume structure and time-averaged diameter appeared similar to that commonly observed in larger wind tunnels. Through preliminary experimentation, the best alignment of the

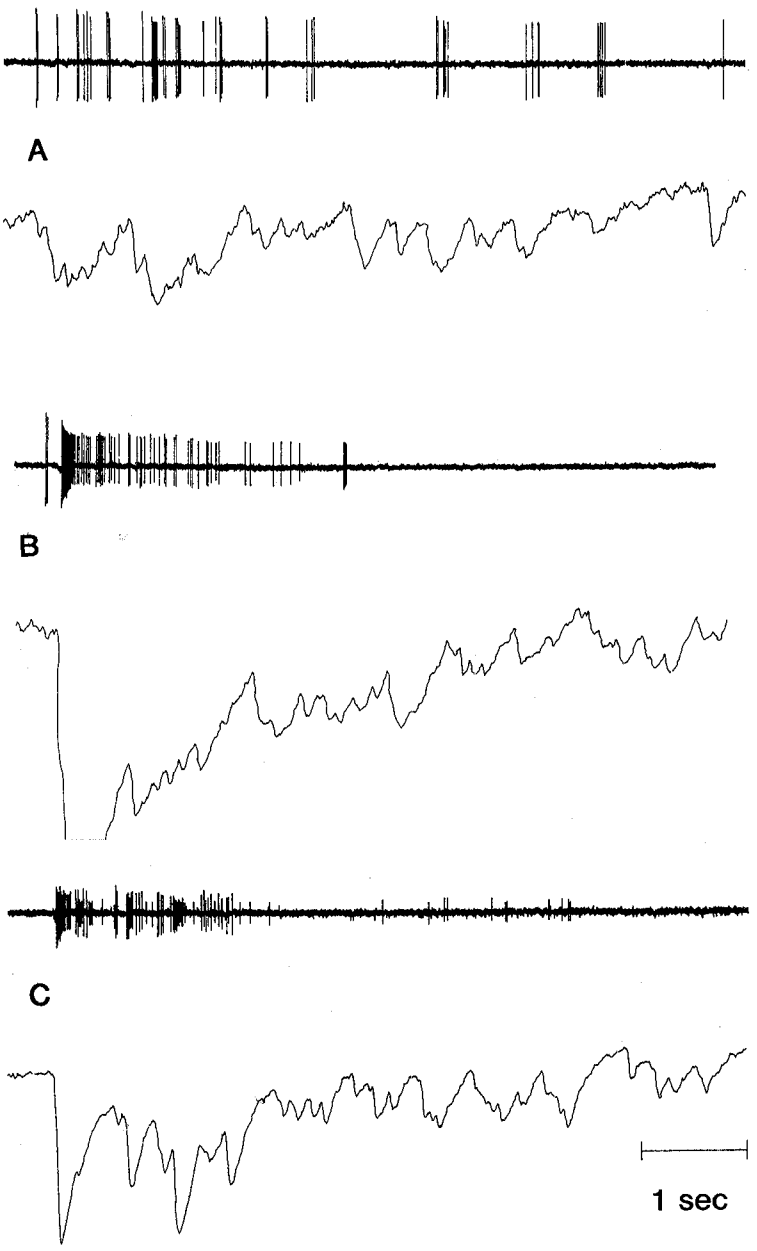

Fig. 1. Action potentials (top traces) and simultaneously recorded DC potentials (bottom traces) of Z5-10:Ac-sensitive neurons (large spikes) on male *A. segetum* antennae placed 70 cm downwind of a rubber septum containing the sex pheromone blend. **(A)** Response during the first few seconds of exposure to a plume from a 30 μg source. **(B)** Response of the same neuron during the first few seconds of exposure to a plume from a 300 μg source. **(C)** Response of a different neuron during the first few seconds of its exposure to a 300 μg source. In this sensillum, a second, non-pheromone-component-sensitive cell (small spike) is also firing.

septum with the antenna for optimal receptor activity was found. The neuronal response was recorded for ~20-s, the source removed and replaced with the next-higher dosage following a 30-s period of clean air. This process was repeated until the antenna had been exposed to the highest concentration. The tip of a new sensillum on the same antenna was then cut, and the neuron recorded from in the same fashion. No more than two sensilla were recorded from on the same antenna. For *A. segetum*, recordings were made from cells responsive to Z5-10:Ac and Z7-12:Ac, and for *H. virescens* only from Z11-16:A1d-sensitive cells.

For *H. virescens*, the same rubber septa used to stimulate antennal neurons were also used to evoke upwind flight in males. The males were released individually into a pheromone plume at the downwind end of a 1 × 3.5 × 0.9 m wind tunnel under conditions described previously that are known to promote optimal flight responses in males (Vetter and Baker, 1983). The temperature was 22°C, light intensity was 0.3 lux, and wind velocity was 0.5 m/s. Males were tested 4–6 h after lights-off on a 16:8 L:D photoperiod regime, and scored for their success at locating the source once they had initiated upwind flight in the plume beginning 3 m from the source, which was placed 15 cm above the floor on a metal platform. Treatments were presented in a randomized, complete-block design, and the platform and septum were replaced after five moths had been tested for their response to a particular dosage.

Results

In a previous study, *A. segetum* males were shown to exhibit upwind flight all the way to rubber dispenser sources containing moderate pheromone dosages (3–30 μg), but terminated upwind flight in the plume if the dosage was 300 μg (Löfstedt *et al.*, 1985). When we exposed single neurons to such plumes 70 cm downwind of the source, they maintained their levels of bursting with the arrival of each pheromone filament for >30 s to the 3 and 30 μg dosage, but adapted within 3–5 s to the 300 μg plume (Figures 1 and 3). Not all of these Z5-10:Ac-sensitive cells became adapted to filaments from the 300 μg source, but the majority, 30 out of 32 cells, did adapt. Our working definition of adaptation in these plumes is a >95% reduction in action potential frequency from the first 1-s period of action potential bursting to subsequent 1-s intervals. Adaptation also was often characterized by a reduction in spike amplitude (Figure 1B,C). The cells recovered after a few minutes of clean air, but rapidly adapted again when the 300 μg source was reintroduced.

The difference between non-adapted *A. segetum* cells at lower concentrations and adapted cells at the highest concentration is actually under-represented in Figure 3 due to the need to average the spike frequencies over each 1-s interval following exposure to pheromone. Especially at the 30 μg dosage, the action potentials came in bursts associated with the arrival of pheromone filaments at a rate of between 1 and 3/s (Figure 1). If there would have been a reliable way to take an average across antennae of the spike frequencies in each burst over time, the spike activity of the cells in response to the lower concentrations compared to the adapted cells would be seen to be even greater. The apparent frequency of filament arrivals in the set-up used for *A. segetum* is in accordance with the rate of filament arrivals on the antennae of *Grapholita molesta* and *Antheraea polyphemus* at similar wind velocities in a wind tunnel, according to

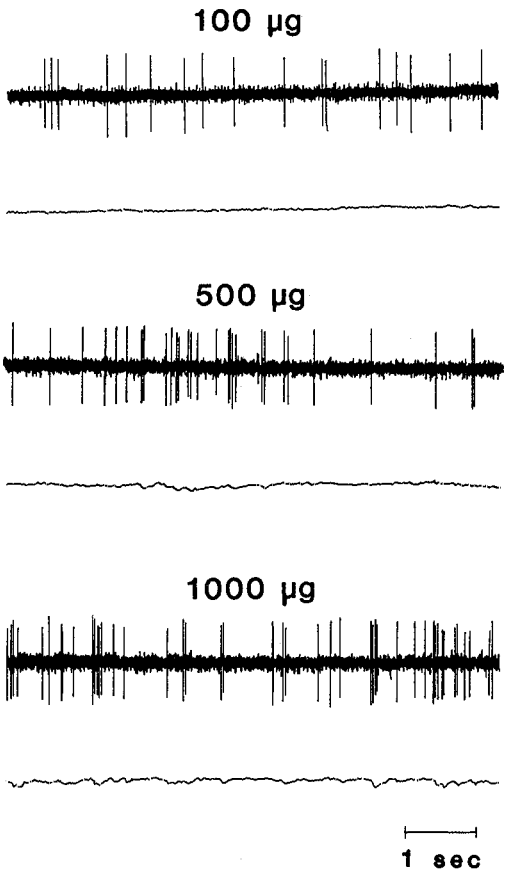

Fig. 2. Action potentials (top traces) and simultaneously recorded DC potentials (bottom traces) of Z11-16:A1d-sensitive neurons on male *H. virescens* antennae placed 70 cm downwind of a rubber septum impregnated with the sex pheromone blend at the indicated loading of Z11-16:A1d.

electroantennogram depolarizations (Baker and Vogt, 1988; Baker and Haynes, 1989).

Also of interest in this experiment is that only five out of 12 cells sensitive to a second component, Z7-12:Ac, became adapted. This suggests that arrestment in this species may be due not only to antennal neurons adapting and sending a false signal of reduced *overall* concentration to the CNS, but, in addition, the signal sent to the CNS might now be erroneous in terms of the *blend ratio* that is actually present. In other words, at high concentrations, the disproportionate adaptation of Z5-10:Ac-sensitive cells compared to those sensitive to Z7-12:Ac might be registered as a change in blend ratio in the CNS when no such change occurred in the filaments contacting the antenna. Ratios of action potentials from different cell types may be used by higher-order neurons for discriminating pheromone blend quality (Kaissling, 1987).

For *H. virescens*, when Z11-16:A1d-sensitive antennal neurons were placed in a plume from sources emitting increasing concentrations of its pheromone blend, no adaptation was observed (Figure 2). At none of the dosages did the action potentials appear to come

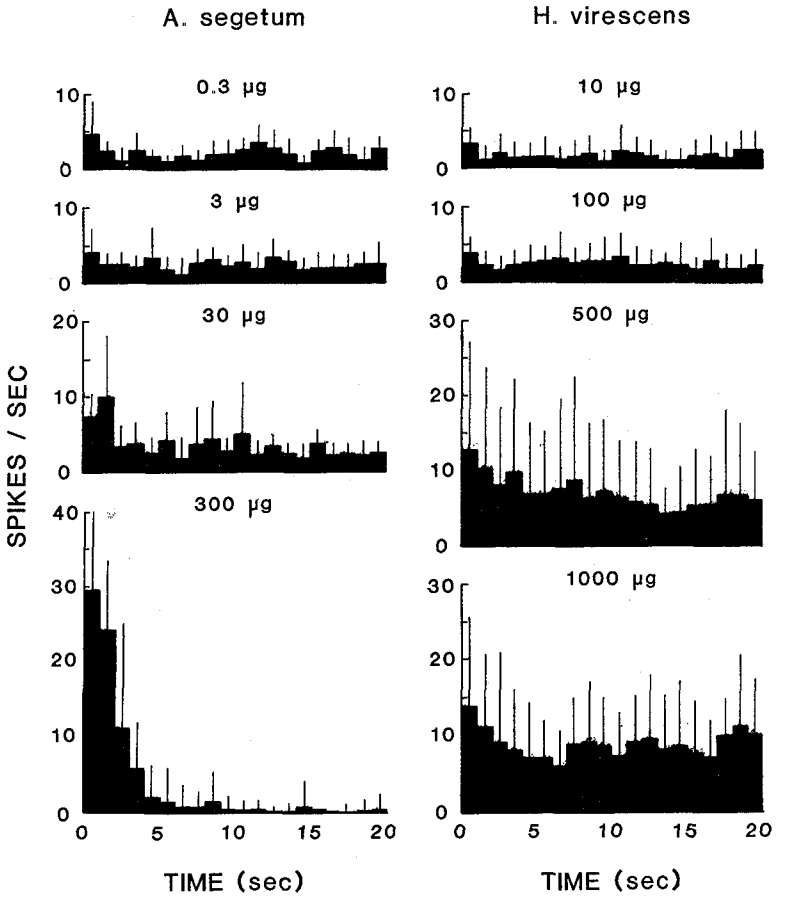

Fig. 3. Mean action potential frequencies of antennal neurons of *A. segetum* sensitive to Z5-10:Ac (n = 32) and of *H. virescens* sensitive to Z11-16:Ald (n = 10) during the first 20 s after being placed directly into a pheromone plume, 70 cm downwind from sources of varying dosages. The first bursts of action potentials in the first few seconds of exposure to the plume were of the highest frequency to the highest dosages for both species, but the only neurons to adapt were *A. segetum's* to 300 µg of its pheromone (lower left). This is a dosage of this blend which results in nearly 100% arrestment of upwind flight (Löfstedt et al., 1985). In contrast, regardless of dosage, *H. virescens'* neurons continued to fire at nearly their initial frequency throughout the first 20 s of exposure, more than enough time for a male to locate the source by means of upwind flight from 3 m downwind. Black vertical lines denote standard deviations.

in bursts as they did in *A. segetum* in response to the 30 µg septum, but rather they were generated in a more uniform spacing over time (Figure 2). This could be another indication that the cells had been stimulated only by moderate concentrations, because Kaissling (1986) found that for *A. polyphemus* the antennal neuronal responses became more phasic and therefore exhibited sharper bursts in response to puffs of the highest concentrations.

The emission rate of the major component Z11-16:Ald from a rubber septum loaded with 100 µg is approximately the same as that from an extruded gland from a calling female, 1–2 ng/min (Vetter and Baker, 1983). Even at the highest source loading of

1000 μg, it is unlikely that we would be able to attain an emission rate of this compound that would challenge the *H. virescens* antennal neurons to the same degree as the neurons on *A. segetum* antennae were challenged by a 300 μg source of its pheromone, due to the higher volatility of the latter. Using the half-life values of decyl acetate from a rubber septum (monounsaturated acetates are nearly identical to saturated) (Butler and McDonough, 1979), the 300 μg *A. segetum* septum should emit ~20 ng/min of Z5-10:Ac.

For behavioral tests of *H. virescens* males in the wind tunnel, we used the same rubber septa that we used for stimulating the antennal neurons. Their non-excessive emission rates were confirmed behaviorally by flying males, because no significant arrestment occurred. Seventy-seven, 89 and 84% of males beginning upwind flight in the plume reached the sources containing 100, 500 and 1000 μg respectively (n = 39, 45 and 43).

Discussion

Our findings draw attention to the capacity of the peripheral olfactory system's ability to handle high-amplitude fluctuating input as a key, initial determinant of whether or not upwind flight in the plume will be sustained or will cease. They also offer a specific, novel explanation as to how the processing of odor quality and quantity could be intimately intertwined; two different component-specific receptors may adapt at different rates when in the same excessive-concentration pheromone plume. The ratio of action potentials from the two cells in response to such a high concentration would be different from their ratio to a lower, optimal concentration of the same blend. Thus, two different concentrations of pheromone, one excessive, might evoke significantly different sensations of odor quality, as previously suggested by Kaissling (1987). This would be especially so when there is a great disparity in the abundance of major and minor pheromone components in airborne pheromone blends, as is so often the case (e.g. Roelofs and Brown, 1982). This disparity would promote the differential adaptation of receptors specific for the major component in a blend when the total blend concentration is high.

Some deutocerebral pathways in moths *depend* on a blend of the correct quality for accentuating signal intermittency. The onset and offset of each pheromone pulse are highlighted by the integration of nearly simultaneously arriving inhibitory and excitatory input from antennal neurons (Christensen and Hildebrand, 1987, 1988). Thus, adaptation which interferes with blend quality may also interfere with the intermittency needed by some CNS circuits. There is apparently a variety of signal-processing pathways in the CNS of *Manduca* (Christensen and Hildebrand, 1988) and *Heliothis* (Christensen *et al.*, 1989), some sensitive to intermittent stimuli, others not.

Should attenuation of the fluctuating neuronal output start to occur as a flying moth advances towards the source, one effect, besides the above-mentioned alteration in blend ratio and odor quality, conceivably would be that the pheromone concentration would appear to diminish. Farther downwind, where a similarly reduced level of fluctuating antennal output *precedes* higher levels of output, the result is rapid upwind progress due to higher airspeeds and lower frequencies of zig-zagging (Kuenen and Baker, 1982). However, should such reduced antennal output follow previously high levels of firing, the effect might be the same as if the moth had experienced a decrease in concentration,

which into clean air results in rapid cessation (beginning within 0.15 s for *G. molesta*) of upwind progress and casting flight (Baker and Haynes, 1987).

In *G. molesta*, attenuation of the fluctuating output of antennal neurons due to partial adaptation occurs when the neurons are challenged by experimentally generated pulse frequencies of 2/s or greater, especially when the neurons are slightly chilled (Baker *et al.*, 1988). This attenuation is also linked to cessation of upwind flight because males of this species are more likely to become arrested in higher concentration plumes at slightly cooler compared to warmer temperatures (Linn *et al.*, 1988). The frequency with which a male's antennae will contact filaments of pheromone in a behaviorally active plume is thus far known to vary from ~0.3 to 2/s, with a typical frequency of 1/s depending on the moth's airspeed, both in the field (Baker and Haynes, 1989) and in the laboratory (Baker and Vogt, 1988; Baker and Haynes, 1989).

Thus, in contrast to the model of Rumbo (1983), which links adaptation to the *promotion* of upwind progress, our results are consistent with an interpretation that there is an association between sensory adaptation and the *failure* to reach the source. It is possible that the CNS could adjust its sensitivity to the reduced input along a component-specific pathway and thus create an adequate signal despite severe receptor adaptation. It is also possible that there is a spectrum of receptors with differing sensitivies to concentration, and that some would only *begin* to become stimulated at high concentrations, exactly when the high-sensitivity cells might be adapting.

Such a situation has been nicely demonstrated in the antennal neurons of *Trichoplusia ni* that are sensitive to the major pheromone component of this species. The dynamic range of sensitivity afforded the moths for this component might ameliorate the effects of adaptation (were it occurring) in the population of cells that is most sensitive to low concentrations (O'Connell *et al.*, 1983; Grant and O'Connell, 1986). *T. ni* males do become arrested in significant proportions in response to rubber septum dosages of 1000 μg or greater of Z7-12:Ac (Linn *et al.*, 1984), and it remains to be seen whether adaptation plays any role at all in this behavior.

In *A. segetum*, we do not think that all antennal sensilla necessarily have identical reactions to temporal stimuli. Our sampling of cells was not extensive enough to allow us to make statements about the proportions of cells that are, or are not, designed to send frequency-modulated signals to the CNS, or whether there are low-sensitivity cells that like those in *T. ni*, function best when the concentration is highest (O'Connell *et al.*, 1983; Grant and O'Connell, 1986). Nevertheless, in our study there was a high correspondence between antennal neuronal output and the behavior, which suggests that the output of neurons from which we were recording is important to behavior, regardless of what their final proportion in the antennal population turns out to be.

Acknowledgements

We thank J. Jönsson for constructing the puffing apparatus and the small wind tunnel, R. Vetter for drawing the figures and rearing *G. molesta* and *H. virescens* and Eva Annerfeldt, Carolina Berglund and Ann Sophie Naumman for rearing *A. segetum*. Supported by NSF grant BNS 8310980 (to T.C.B.) and grants from the Swedish Natural Resources Council to J.L., C.L. and T.C.B.

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Received on August 1, 1988, accepted on February 2, 1989