chain homologous to the Con A–binding glycoproteins.

Since the synapses from which these glycoproteins originated were isolated from whole brain, one type of glycoprotein may be associated with a particular class of synapses, or these glycoproteins may have different functions at the same synapse. A means of recognizing specific glycoproteins at particular synapses appears to be necessary in order to distinguish between these possibilities and to further elucidate the function of these glycoproteins in the establishment and maintenance of synaptic contacts.

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Pheromone Source Location by Flying Moths: A Supplementary Non-Anemotactic Mechanism

Abstract. *After the wind was stopped in an insect flight tunnel, male oriental fruit moths continued to fly in zigzag fashion along a stationary pheromone plume. Their lateral excursions from the time-averaged pheromone plume were no greater without wind than in wind of 38 centimeters per second. When the pheromone plume was removed and the wind stopped, males initiated wider track reversals when they reached the pheromone-free area in still air than they had made while in the pheromone plume. This non-anemotactic mechanism of maintaining plume contact—possibly a special kind of klinotaxis—when coupled with the orthokinetic retinal velocity of apparent ground pattern motion, allowed males to reach the pheromone source area from 1 to 2 meters away without wind.*

Optomotor anemotaxis is an important mechanism in the successful location of pheromone sources by flying moths (*1*, *2*). Such pheromone-mediated steering with respect to the wind by using the motion pattern of cues from the visual field has often been considered to be the only mechanism involved in pheromone source finding. However, other mechanisms, such as orthokinesis, must occur in conjunction with steering to propel the moth over the ground toward the source (*3*). Farkas and Shorey (*4*) proposed that "aerial trail following" to maintain lateral contact with the plume occurred by means of a chemotactic turning back toward the plume; they implied that a longitudinal effect also occurred to allow males to arrive at the more concentrated area of pheromone near the source. In still air with an intact plume, pink bollworm males could successfully orient to near the pheromone source (*4*). Kennedy and Marsh (*1*) disproved the longitudinal aspect of their hypothesis by demonstrating that progress toward a pheromone source requires an optomotor feedback response to the apparent movement of the visual environment and that the males in the Farkas-Shorey experiments had already set their optomotor anemotactic courses and orthokinetic velocities before the wind stopped. To end up near the source, the moths only had to retain their general up-tunnel direction by maintaining for a short time the optical sensation of front-to-back ground pattern movement developed while they were in the wind.

We reexamined the problem of orientation to pheromone in a windless environment, focusing on the lateral component of movement that might keep males close to the plume. This type of movement has not been thoroughly examined by previous researchers (*1*, *2*, *4*). We found that males can indeed locate a pheromone source with a high success rate without the aid of wind, provided they have already established a set ground speed and direction while in the wind. We also found evidence that when the wind dropped to zero, lateral contact with the (time-averaged) plume is maintained without the aid of anemotaxis and possibly occurs through a protracted chemoklinotaxis. The wide casting movements characteristic of plume loss, called reversing anemomenotaxis when there is wind (*1*, *2*), also occur upon plume loss when there is no wind. This finding further supports a chemotactic mechanism for maintaining lateral contact with the plume.

Oriental fruit moth males at their time of peak sexual activity (*5*) were released individually in the pheromone plume from a small screen cage at the downwind end of a wind tunnel (working area, 3.7 by 1.0 by 0.9 m) (*6*). The synthetic sex pheromone source (*7*, *8*), a rubber septum impregnated with the three sex attractant components for this species, was placed on a sheet metal platform (15 by 15 by 15 cm) at a location 3.1 m up-tunnel from the males. We first tested to see whether males could locate the source without wind by allowing them to begin up-tunnel flight in the (time-averaged) plume. We then quickly stopped the wind (velocity, 38 cm/sec) (*9*). The males could locate the source much of the time without wind (44 percent, $N = 27$) and a large percentage (81 percent) could get within 10 cm of the septum, averaging a flight of 1.5 ± 0.4 m [mean ± standard deviation (S.D.)] ($N = 22$) after wind stoppage to do so. This confirms the unquantified results of a similar experiment on *Pectinophora gossypiella* (*4*). None of the males that flew to within 10 cm from the source in zero wind flew beyond it; rather, half actually landed on the platform and displayed hairpencils at the septum. If only up-tunnel momentum were involved, the moths should have proceeded past the source. The mean distance from the plume's axis during track reversal (turning back toward the plume) was not significantly greater for moths flying without wind (6.6 ± 1.9 cm, $N = 27$)

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than for those flying with wind (5.5 ± 2.6 cm, $N = 42$; t-test, $P > .05$), indicating that flight accuracy along the plume was not reduced in the absence of wind (Fig. 1A) (*9*). Ground velocity up-tunnel was maintained by moths flying in zero wind [44.2 ± 25.1 cm/sec] relative to their previous velocity of 30.0 ± 14.0 cm/sec. Also, the mean ± S.D. of the interreversal track angles (*2, 9*) in wind and after the wind stopped were similar, 57.8° ± 12.4° and 51.9° ± 14.6°, respectively, for these moths ($P > .05$, t-test).

Because anemotaxis could no longer be operating after wind stoppage, a steering from a visual memory of the velocity of apparent ground pattern motion and interreversal track angle (*2*) must have taken over, but the similarity of track reversal distances suggested that windless steering might depend on the intact, remaining plume. If so, a form of chemotaxis might be used by moths to maintain plume contact.

In a second experiment, we used a wind velocity of 70 cm/sec with the same procedures as before, except that the rubber septum was suspended by a thread from the tunnel's ceiling and, on signal, was pulled abruptly up to the top. One second later the fan was turned off, and the wind was stopped an average of 2.7 seconds later. This procedure (*10*), verified with smoke, caused the 15-cm-high up-tunnel end of the plume to come to rest near the middle of the camera's field of view. When timed properly (and fortuitously) with a male beginning a flight up the tunnel, this procedure provided recordings of males in zero wind flying up-tunnel out the front end of the pheromone plume into clean air (*10*). Flight tracks of such males changed significantly as the males reached the clean-air region (Fig. 1B) in that track reversals occurred significantly farther from the plume axis (11.5 ± 3.4 cm, $N = 13$) than they had in the plume area (6.8 ± 2.5 cm, $N = 13$; t-test, $P < .001$). Track reversals of males in zero wind when the plume was sham-removed occurred at 7.5 ± 2.0 cm ($N = 19$) and 7.7 ± 4.2 cm for the pheromone plume and "clean-air" areas, respectively (not significantly different; t-test, $P > .05$). In addition, 43 percent ($N = 23$) of the males flew to within 10 cm of the source without wind when the plume was sham-removed (mean closest approach, 29.1 ± 35.7 cm), whereas no males ($N = 24$) flew to within 10 cm in zero wind when the plume was removed (mean closest approach, 97.6 ± 39.0 cm). Interreversal track angles were significantly greater (more perpendicular to the plume axis) for males in the nonplume area (82.8° ± 19.7°, $N = 13$) compared to those in the same area in the sham-removal situation (62.7° ± 17.5°, $N = 18$) or in the plume area before flying into clean air (59.5° ± 15.6°, $N = 13$) (t-test, $P < .01$) (*11*). These changes in track could not have been steered anemotactically because there was no wind.

A final experiment demonstrated again the males' tendency to keep their excursions close to the time-averaged plume regardless of wind velocity. This time the naturally flowing plume in moving air was maintained while the airspeed along the windline was reduced to nearly 0 cm/sec by moving the males down-tunnel by means of a fast-moving striped ground pattern (*12*). Whether drifting down-tunnel at close to the prevailing wind velocity of 37.5 cm/sec or flying up-tunnel at higher airspeeds against the wind, track reversals occurred at the same average distance from the plume axis (Figs. 1C and 2). Males flying down-tunnel faster than the wind (negative values in Fig. 2) also tended to keep their track reversals close to the plume axis. Moreover, after the plume was removed (*12*), significantly wider track reversals occurred, whether males were drifting down-tunnel at an average airspeed of about 0 cm/sec (Figs. 1D and 2) or at higher airspeeds by

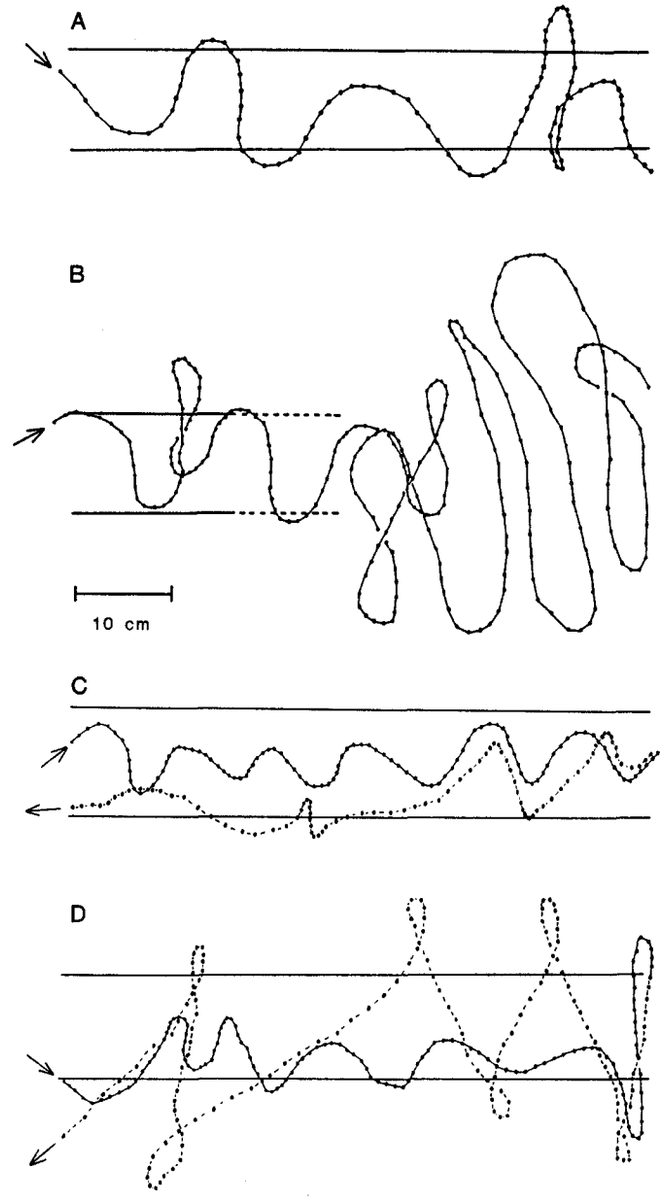

Fig. 1. Plan view (from above) of flight tracks of male oriental fruit moths through a 65-cm-long section of wind tunnel. Dots represent each 1/60 second of elapsed time. Solid straight lines denote the boundaries of the time-averaged pheromone plume as visualized by smoke. The pheromone source was 105 cm from the right edge in each illustration, and the wind, when present, was from the right. Arrows denote the direction of flight in each track. (A) The wind stopped 0.25 second before the male entered the field of view, and the male continued to the septum. (B) The pheromone plume was removed, and the wind stopped just as the male entered the field of view; the approximate up-tunnel end of the plume (by smoke visualization) is indicated by the dashed, straight lines; on entering the nonplume area the male's track changed significantly, with a closest approach to the septum of 90 cm. (C) Wind was present at 37.5 cm/sec; the male was allowed to fly up-tunnel (solid track) and was then brought back down-tunnel (dashed track) at an average velocity of 42.9 cm/sec with a moving floor pattern, for an average airspeed along the windline of −5.4 cm/sec. Track reversal distances remained small with continued plume contact both at high and low airspeeds (up- and down-tunnel). (D) At a wind velocity of 28.6 cm/sec, the plume was removed, and the male flying up-tunnel (solid track) reached the plume's up-tunnel terminus at the right edge of the field of view; the male was then brought back down-tunnel in pheromone-free air (dashed track) with a floor pattern velocity matching that of the wind, and the average airspeed created was −8.0 cm/sec.

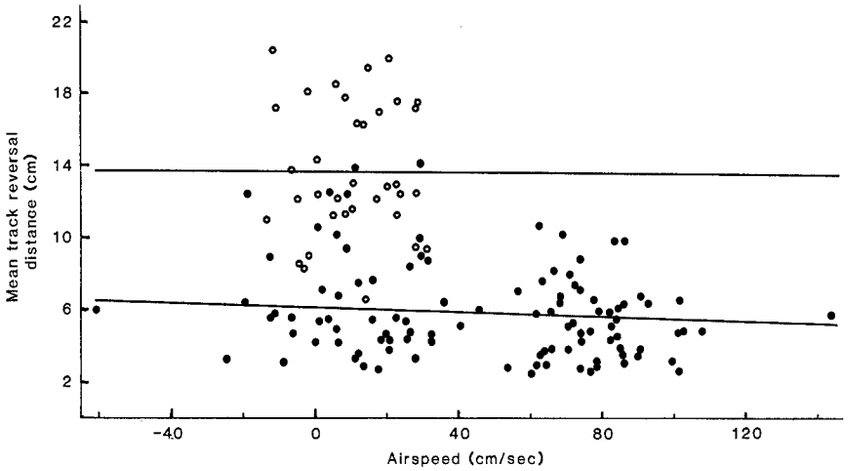

Fig. 2. Correlation between average airspeed along the windline and distances of track reversal from the plume axis (●) when the plume was present ($r = -.192$) and (○) when it had been removed ($r = -.006$).

keeping station with the stationary pattern. The airspeed measurements were time-averaged, and it is possible that quick "samplings" of the wind's direction could have been obtained by the males as they reversed track and briefly slowed their down-tunnel drift (Fig. 1, C and D). Nevertheless, the presence of a naturally moving, structured pheromone plume significantly affected track reversal distances, whereas the males' airspeeds did not.

We propose that in zero wind—although there may be other possible mechanisms (*7*) such as klinokinesis, transverse klinotaxis (*3*), and tropotaxis—the most likely mechanism responsible for males' maintenance of plume contact is longitudinal klinotaxis (*3*), whereby a visual memory of the previous track or pattern of track reversals would permit an oriented (with respect to previous tracks) turn or series of turns to take place, depending on pheromone concentration. Right-left track reversals can occur even in wind permeated with pheromone, with a frequency determined by the pheromone concentration (*13*). Loss of pheromone is followed by larger reversals. We suggest that when wind ceases, the pheromone concentration–mediated frequency of reversals and the angle of interreversal legs, previously steered anemotactically by compensatory adjustment of drift angle and airspeed (*2*), now are steered with respect to the ground pattern alone; in this event, the drift angle in zero wind is reduced to zero, making the course and track identical. Reduction of pheromone concentration in zero wind would decrease the frequency of reversals and increase the angles of interreversal legs. The moth would need to sense only the decrease in concentration with time, not the direction of departure from the plume; sensing the latter would be highly improbable in view of the randomness of the instantaneous structure of the plume and the moth's track relative to it (*3*). To reach the source without wind, the concentration-dependent reversal pattern would need to be coupled with an orthokinetic component regulated by the velocity of an apparent front-to-back ground pattern movement whose up-tunnel polarity was developed during anemotaxis. In our experiments, ground velocities up-tunnel with and without wind were similar and resulted in displacement toward the source. It appeared that those males making more rapid progress up-tunnel while in the wind were more likely than the more stationary males to arrive near the source after the wind was stopped.

Kennedy (*3*) considered longitudinal klinotaxis to be a mechanism by which flying male moths could locate pheromone sources under windless conditions. At least one moth species, and possibly two (*4*), appear to use this mechanism immediately after anemotactically guided flight in the presence of pheromone. Because optomotor anemotaxis must precede the chemotaxis, it would operate only transiently and would not in itself be enough to guide the male all the way to a female, as had been proposed earlier (*4*), and we have no evidence that it would operate in place of anemotaxis in the presence of wind (*4*). Our proposed chemoklinotactic mechanism differs substantially from that proposed earlier (*4*) in that the turns and lateral movements made in still air that duplicate those made while wind was present are not specifically back toward the plume axis. Rather, the turns would be part of a protracted program of left-right track reversals that are narrow in the presence of pheromone and wide in its absence (*2, 13*) (Fig. 1B).

Under natural conditions in the field, it is conceivable that wind velocity could subside to zero either at the time of a male's arrival to within a few meters of a calling female (as in our wind tunnel) or at any point during a previously anemotactically guided flight. The female, whose position is always correctly indicated by the combination of wind direction plus pheromone (*14*), would be located more rapidly by males who could, during such wind lulls, continue their previous flight track pattern for some distance in the continued presence of pheromone.

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9. One person turned off the fan and stopped the blades by pulling on a leather cinch wrapped around the drive axle. A second person at the downwind end simultaneously covered the exhaust tube with a large piece of cardboard, and a third person at the tunnel's center, upon giving the command to stop the fan, flashed a tiny, light-emitting diode on top of the tunnel in the camera's field of view and again upon the instant the fan blades stopped moving. A TiCl/4 smoke source on a septum, creating a time-averaged plume (diameter, 10 cm) in the tunnel's recording section, verified that the wind had stopped completely at the moment the fan's blades stopped and that the smoke plume retained its general shape for about 10 seconds after wind cessation. However, gradual expansion and dissipation of the plume began 2 to 3 seconds after the wind was stopped. Flight tracks were video-recorded from above in a plan view with a rotary-shutter camera (Sony RSC 1050) located on top of the tunnel and a recorder (Sony SLO 340). The field of view for all recordings extended 65 cm, from 105 to 170 cm down-tunnel from the pheromone source and about 40 cm across the tunnel. Tracks were recorded onto a motion analyzer (Sony SVM 1010) played back frame by frame, and the moths' locations each 1/60 second were traced onto acetate sheets. The distances of each moth's track reversals from the plume axis were measured with an X/Y digitizer (Houston Instruments). Most reversals took the moths back toward the plume axis, but a small percentage were away from the axis and were not used in analysis. Angles of the interreversal tracks [the fairly straight runs with a large cross-tunnel component (*2*)] were measured by hand with a protractor, 0° being direct-

ly up-tunnel and 90° being perpendicular to the plume axis both to the right and left.

10. The light-emitting diode was flashed at the instant the plume was removed and again when the fan blades had stopped to indicate the moment of wind stoppage. The smoke source visualization resulted in the plume's up-tunnel end coming to rest about 30 cm from the down-tunnel field of view, with an average time of 3.7 seconds from plume removal to wind stoppage. The average stoppage time for all experimental trials was 3.7 ± 0.2 seconds with a wind velocity of 70 cm/sec (N = 13). The slight variation in stoppage time created a bias against finding differences in track reversal distance because the presence of a plume in the "nonplume" section and vice versa would have influenced reversal magnitude, blurring the differences between turning in the two areas.
11. Ground velocities up-tunnel were generally lower in the nonplume area (26.3 ± 23.5 cm/sec, N = 13) than they were in the plume area (45.6 ± 26.2 cm/sec, N = 13) in the plume-removal situation or than they were in the nonplume area in the sham-removal situation (40.9 ± 23.8 cm/sec, N = 18), although not significantly (t-test, $P > .05$).
12. Males were allowed to fly up-tunnel through the recording section and then were brought back down-tunnel at near zero airspeed along the windline with the plume present by moving the ground pattern down-tunnel at a velocity greater than the wind velocity. For trials where the plume was removed, the male was allowed to fly into the recording section and the plume was then removed, so that loss of pheromone occurred near the section's up-tunnel end. The ground pattern's velocity down-tunnel was matched exactly to that of the wind so that males keeping station with the stripes would drift down-tunnel at an effective wind velocity near zero.
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Experimental Hepatic Encephalopathy: Changes in the Binding of γ-Aminobutyric Acid

Abstract. *Two populations of receptors for γ-aminobutyric acid, one with low- and the other with high-affinity characteristics, are detectable in frozen, thawed, Triton-treated synaptic membrane preparations from normal brain. It is now reported that membrane preparations from rats with mild galactosamine-induced hepatic encephalopathy show an increase in the number of low- and high-affinity γ-aminobutyric acid binding sites, whereas those from rats with severe encephalopathy show only high-affinity binding sites. Thus, hepatic encephalopathy appears to involve partial degeneration of the γ-aminobutyric acid–containing presynaptic nerve terminals.*

Hepatic encephalopathy (HE) following fulminant hepatic failure (FHF) occurs when overall liver function is grossly subnormal as a consequence of massive necrosis of the liver. Despite the several metabolic abnormalities described in this situation, the mechanism of the central nervous system (CNS) derangement in the onset of coma is still unknown (*1*). A critical review of the studies of this pathology led us to consider that, whatever pathogenetic factors cause the encephalopathy in FHF, little is known about the functional activity of γ-aminobutyric acid (GABA) receptors in the CNS of animals with FHF. In fact, changes in the function of GABA receptors have largely been ignored in considering the mechanism underlying the neurological disturbances of HE.

A suitable animal model of FHF, that closely resembles the human FHF in both histological lesion and metabolic changes, was obtained by injecting rats with the selective hepatotoxin D-galactosamine hydrochloride (*2, 2a*). Approximately 70 percent of rats so injected develop FHF with an encephalopathy characterized by increasing stupor and flaccidity, poor righting reflex, and diminished response to pain; deep coma develops at 3.5 to 4 days. From the mild stage of HE some of the rats recover as a result of liver regeneration (*3*).

Rats with mild and severe HE were evaluated by means of visual evoked potentials (VEP) as described (*3*) so that we could select for binding studies the brains of animals with the same degree of HE. In male Sprague-Dawley rats (100 to 125 g), two cortical electrodes were implanted through the skull in contact with the cortex and permanently fixed with dental cement under ethyl ether anesthesia. Three days after the surgical procedure a control VEP was recorded just before intraperitoneal injection of D-galactosamine hydrochloride (3 g/kg). Subsequently, VEP's were recorded at different times during the development of HE. For the binding studies, rats in mild and severe stages of HE were killed by decapitation and their brains were removed and frozen.

We performed the binding studies with fresh-frozen and frozen Triton X-100–treated membranes from the whole brains of 23 control rats and 23 rats with severe HE, using tris-citrate buffer (*p*H 7.1) and tritiated GABA as described by others and by us (*4, 5*). For studies with the fresh-frozen synaptic preparations, we thawed portions of the frozen homogenates at room temperature and washed them two times with tris-citrate buffer just before the assay, using approximately 300 to 400 μg of proteins per milliliter (*6*). The remaining frozen homogenates were thawed and treated three times with Triton X-100 and extensively washed. The Scatchard plots of six different saturation curves obtained for fresh-frozen membranes from three to four pooled brains revealed the presence of one population of GABA receptors in both the control and comatose rats. However, the mean (±1 standard deviation) of the affinity constants (K_d) of GABA binding to membranes from HE rats (K_d = 90 ± 7 nM) was significantly increased in comparison with that of controls (K_d = 187 ± 14 nM) (Student's t-test, $P < .001$), whereas the maximum binding (B_{max}) was decreased (2.2 ± 0.3 as opposed to 4.4 ± 0.5 pmole per milligram of protein) ($P < .01$), suggesting a reduction of endogenous inhibitors of GABA receptors and a loss of binding sites.

These differences in the GABA bind-

Table 1. Kinetic constants of Na^+-independent [^{3}H]GABA binding in membranes prepared from brains of normal rats, from brains of D-galactosamine–injected rats that did not develop FHF (no HE), and from D-galactosamine–injected rats that developed mild and severe HE. The binding was performed as described in Fig. 1. The reported values represent the means (± 1 standard deviation) of the kinetic components computed from the Scatchard plots obtained from six separate experiments done in triplicate; each experiment was performed on membranes prepared from a pool of three to four brains. Statistical significance was determined by Student's t test.

Rats	Total binding sites	[^{3}H]GABA kinetic components				$\frac{B_{max2}}{B_{max1}}$
		Low affinity		High affinity		
		K_{d1}*	B_{max1}†	K_{d2}*	B_{max2}†	
Controls	7.3	218 ± 15	6.0 ± 0.9	19.6 ± 6	1.3 ± 0.3	0.21
D-Galactosamine treated						
No HE	7.4	240 ± 20	6.0 ± 0.5	18.6 ± 3	1.4 ± 0.2	0.23
Mild HE	10.0	311 ± 6	8.1 ± 0.2‡	24.0 ± 4	1.9 ± 0.2§	0.23
Severe HE	1.9	‖	‖	22.9 ± 4	1.9 ± 0.1§	0

*Measured as nanomolar concentrations. †Measured as picomoles per milligram of protein. ‡$P < .05$. §$P < .01$, compared to controls. ‖ Binding sites undetectable.

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